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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Ornithobacterium rhinotracheale subunit vaccines

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Ornithobacterium rhinotracheale subunit vaccines.

The present invention relates to nucleic acids encoding *Ornithobacterium rhinotracheale* proteins, to DNA fragments, recombinant DNA molecules, live recombinant carriers and host cells comprising such nucleic acids, to *Ornithobacterium rhinotracheale* proteins, to antibodies against such proteins, to such proteins for use in vaccines, to the use of such proteins in the manufacturing of such vaccines, to vaccines comprising such nucleic acids, DNA fragments, recombinant DNA molecules, live recombinant carriers, host cells, proteins or antibodies against such proteins, and to methods for the preparation of such vaccines.

Ornithobacterium rhinotracheale is a relatively recently discovered bacterium that is found more and more frequently in poultry farms, and in wild birds. Especially animals in commercial chicken farms, turkey farms and duck farms are frequently infected.
In commercial poultry, infection is associated with respiratory diseases: airsacculitis and pneumonia are the most common features of infection with *Ornithobacterium rhinotracheale*. These signs can be induced by aerosol in intra-tracheal or intra-thoracic administration of the organism and are aggravated by other factors such as respiratory viruses, bacteria or sub-optimal housing conditions. Osteitis, meningitis and joint-infections which can be induced by intravenous application have been associated with *Ornithobacterium rhinotracheale*. The infection can be transmitted horizontally, as well as vertically through eggs, which probably accounts for its rapid and worldwide spread. An extensive review of *Ornithobacterium rhinotracheale* has been given by van Empel, P.C.M. ad Hafez, H.M. in Avian Pathology 28:217-227 (1999). European Patent EP0.625.190 relates to both the *Ornithobacterium rhinotracheale* bacterium and to vaccines against *Ornithobacterium rhinotracheale.*

Serological research has revealed that *Ornithobacterium rhinotracheale* strains may have different serotypes, to a certain degree depending on the geographic origin of the strain and the host animal from which they were isolated. At this moment, eighteen different serotypes are found.

Therapeutic treatment of the disease can be difficult because acquired resistance against the regular antibiotics is very common within the genus. Moreover, there is an increasing reluctance against the use of antibiotics in food animals for both public health- and environmental reasons.

Vaccination offers an alternative for therapeutic treatment with antibiotics, but up till now, only vaccination with live attenuated vaccines and inactivated whole cell vaccines was possible.

The success of live attenuated vaccines specifically for *Ornithobacterium rhinotracheale* depends highly on the right balance between attenuation and triggering of the immune system. Inactivated whole cell vaccines are basically safe and therefore, from a safety point of view would seem the preferred type of vaccine.

Inactivated whole cell vaccines however need to be given in a higher dose compared to live attenuated vaccines. As a general rule, most of the proteins present in a bacterium play no role in the triggering of the immune system, i.e. they are not relevant immunogens. This means that, in the case of inactivated whole cell vaccines, in order to provide humans or animals with a sufficient level of relevant immunogens a lot of non-protective material is additionally and unavoidably administered. This is not a desirable situation.

The use of subunit vaccines could overcome this problem, and would therefore be highly preferred, but currently no immunogenic subunit vaccines are known in the art for combating *Ornithobacterium rhinotracheale*.

Moreover, although live attenuated vaccines and inactivated whole cell preparations are known to provide a certain level of cross-protection against all *Ornithobacterium rhinotracheale* strains, subunit vaccines might or might not induce cross-reactivity.

The present invention aims at providing for the first time vaccines that are based upon *Ornithobacterium rhinotracheale* subunits that do induce cross-reactivity.

This objective is reached by providing eight novel *Ornithobacterium rhinotracheale* proteins that surprisingly play an important role in triggering a protective immune response, and by providing vaccines comprising one or more of these novel immunogenic proteins.
Even more surprisingly, these eight novel proteins were found no only to induce a protective homologous immune response, but to also induce a protective cross-reactive immune response.
A homologous immune response is a response against strains of the same serotype, whereas a cross-reactive immune response is a response against both serologically homologous and heterologous strains.

The first novel protein, Or01, having a molecular weight of 59.8 kD is encoded by a nucleic acid having a nucleotide sequence as depicted in SEQ ID NO: 1.

It is well-known in the art, that many different nucleotide sequences can encode one and the same protein. This phenomenon is commonly known as wobble in the second and especially the third base of each triplet encoding an amino acid. This phenomenon can result in a heterology of about 20-30% for two nucleotide sequences still encoding the same protein. Therefore, two nucleic acids having a nucleotide sequence homology of about 80 % can still encode one and the same protein.

Thus, one embodiment relates to a nucleic acid encoding a 59.8 kD *Ornithobacterium rhinotracheale* protein or a part of said nucleic acid that encodes an immunogenic fragment of said protein wherein said nucleic acid or said part thereof has at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 1.

The molecular weight of the protein (and the seven other proteins) is determined on the basis of the molecular weight of the amino acids as given in the amino acid sequence.

Preferably, a nucleic acid according to the invention encoding this 59.8 kD *Ornithobacterium rhinotracheale* protein or a part of that nucleic acid that encodes an immunogenic fragment of that protein has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 1.

Even more preferred is a homology level of 98 %, 99 % or even 100 %.

The level of nucleotide homology can be determined with the computer program "BLAST 2 SEQUENCES" by selecting sub-program: "BLASTN" that can be found at **www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html.**
A reference for this program is Tatiana A. Tatusova, Thomas L. Madden FEMS Microbiol. Letters 174: 247-250 (1999). Parameters used are the default parameters:
Reward for a match: +1. Penalty for a mismatch: -2. Open gap: 5. Extension gap: 2. Gap x_dropoff: 50.

Another approach for deciding if a certain nucleic acid sequence is or is not a nucleic acid sequence according to the invention relates to the question if that certain nucleic acid sequence does hybridize under stringent conditions to the nucleotide sequence as depicted in SEQ ID NO: 1 (or in SEQ ID NO: 3, 5,7, 9, 11, 13 or 15,. see below).

If a nucleic acid sequence hybridizes under stringent conditions to the nucleotide sequence as depicted in SEQ ID NO: 1, or of course as depicted in SEQ ID NO: 3, 5,7, 9, 11, 13 and 15, it is considered to be a nucleic acid sequence according to the invention.

The definition of stringent conditions follows from the formula of Meinkoth and Wahl (1984. Hybridization of nucleic acids immobilized on solid supports. Anal. Biochem. 138: 267-284.).

Tm = [81.5°C + 16.6(log M) + 0.41(%GC) - 0.61(%formamide) - 500/L] - 1°C/1%mismatch

In this formula, M is molarity of monovalent cations; %GC is the percentage of guanosine and cytosine nucleotides in the DNA; L is the length of the hybrid in base pairs.

Stringent conditions are those conditions under which nucleic acid sequences or fragments thereof still hybridize, if they have a mismatch of 20 % at the most, preferably 10%, more preferably 8, 6, 5, 4,3, 2, 1 or 0% in that order or preference, to the nucleic acid sequence as depicted in any of the SEQ ID NO: 1, 3, 5,7, 9, 11, 13 or 15.

Another embodiment relates to a nucleic acid encoding a 58.2 kD *Ornithobacterium rhinotracheale* protein Or02, or a part of said nucleic acid that encodes an immunogenic fragment of said protein wherein said nucleic acid or said part thereof has at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 3.

Preferably, a nucleic acid according to the invention encoding this 58.2 kD *Ornithobacterium rhinotracheale* protein or a part of that nucleic acid that encodes an immunogenic fragment of that protein has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 3.

Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Still another embodiment relates to a nucleic acid encoding a 46.0 kD *Ornithobacterium rhinotracheale* protein Or03 or a part of said nucleic acid that encodes an immunogenic fragment of said protein wherein said nucleic acid or said part thereof has at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 5.

Preferably, a nucleic acid according to the invention encoding this 46.0 kD *Ornithobacterium rhinotracheale* protein or a part of that nucleic acid that encodes an immunogenic fragment of that protein has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 5.

Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Again another embodiment relates to a nucleic acid encoding a 37.2 kD *Ornithobacterium rhinotracheale* protein Or04 or a part of said nucleic acid that encodes an immunogenic fragment of said protein wherein said nucleic acid or said part thereof has at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 7.

Preferably, a nucleic acid according to the invention encoding this 37.2 kD *Ornithobacterium rhinotracheale* protein or a part of that nucleic acid that encodes an immunogenic fragment of that protein has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 7.

Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Another embodiment relates to a nucleic acid encoding a 45.6 kD *Ornithobacterium rhinotracheale* protein Or11 or a part of said nucleic acid that encodes an immunogenic fragment of said protein wherein said nucleic acid or said part thereof has at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 9.

Preferably, a nucleic acid according to the invention encoding this 45.6 kD *Ornithobacterium rhinotracheale* protein or a part of that nucleic acid that encodes an immunogenic fragment of that protein has at least 85 %, preferably 90 %, more preferably 95 % homology with the

nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 9.

Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Again another embodiment relates to a nucleic acid encoding a 42.2 kD *Ornithobacterium rhinotracheale* protein Or77 or a part of said nucleic acid that encodes an immunogenic fragment of said protein wherein said nucleic acid or said part thereof has at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 11.

Preferably, a nucleic acid according to the invention encoding this 42.2 kD *Ornithobacterium rhinotracheale* protein or a part of that nucleic acid that encodes an immunogenic fragment of that protein has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 11.

Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Also another embodiment relates to a nucleic acid encoding a 34.0 kD *Ornithobacterium rhinotracheale* protein Or98A or a part of said nucleic acid that encodes an immunogenic fragment of said protein wherein said nucleic acid or said part thereof has at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 13.

Preferably, a nucleic acid according to the invention encoding this 34.0 kD *Ornithobacterium rhinotracheale* protein or a part of that nucleic acid that encodes an immunogenic fragment of that protein has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 13.

Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Another embodiment relates to a nucleic acid encoding a 32.9 kD *Ornithobacterium rhinotracheale* protein Or98B or a part of said nucleic acid that encodes an immunogenic fragment of said protein wherein said nucleic acid or said part thereof has at least 80 %

homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 15.

Preferably, a nucleic acid according to the invention encoding this 32.9 kD *Ornithobacterium rhinotracheale* protein or a part of that nucleic acid that encodes an immunogenic fragment of that protein has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 15.

Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Nucleotide sequences that are complementary to the sequence depicted in SEQ ID NO 1, 3, 5, 7, 9, 11, 13 or 15 or nucleotide sequences that comprise tandem arrays of the sequences according to the invention are also within the scope of the invention.

Since the present invention discloses nucleic acids encoding 8 novel *Ornithobacterium rhinotracheale* proteins, it is now for the first time possible to obtain these proteins in significant quantities. This can e.g. be done by using expression systems to express the whole or parts of a gene encoding the protein or an immunogenic fragment thereof.

Therefore, in a preferred form of this embodiment, the invention relates to DNA fragments comprising a nucleic acid according to the invention. A DNA fragment is a stretch of nucleotides that functions as a carrier for a nucleic acid according to the invention. Such DNA fragments can e.g. be plasmids, into which a nucleic acid according to the invention is cloned. Such DNA fragments are e.g. useful for enhancing the amount of DNA for use as a primer and for expression of a nucleic acid according to the invention, as described below.

An essential requirement for the expression of the nucleic acid is an adequate promoter functionally linked to the nucleic acid, so that the nucleic acid is under the control of the promoter. It is obvious to those skilled in the art that the choice of a promoter extends to any eukaryotic, prokaryotic or viral promoter capable of directing gene transcription in cells used as host cells for protein expression.
Therefore, a more preferred form of this embodiment relates to a recombinant DNA molecule comprising a DNA fragment and/or a nucleic acid according to the invention wherein the nucleic acid according to the invention is placed under the control of a functionally linked promoter. This can be obtained by means of e.g. standard molecular biology techniques.

(Maniatis/Sambrook (Sambrook, J. Molecular cloning: a laboratory manual, 1989. ISBN 0-87969-309-6).

Functionally linked promoters are promoters that are capable of controlling the transcription of the nucleic acids to which they are linked.

Such a promoter can be the native promoter of the novel gene, i.e. the promoter that is involved in the transcription of the nucleic acid encoding a protein according to the invention, or another promoter of *Ornithobacterium rhinotracheale*, provided that that promoter is functional in the cell used for expression. It can also be a heterologous promoter. When the host cells are bacteria, useful expression control sequences which may be used include the Trp promoter and operator (Goeddel, et al., Nucl. Acids Res., 8, 4057, 1980); the lac promoter and operator (Chang, et al., Nature, 275, 615, 1978); the outer membrane protein promoter (Nakamura, K. and Inouge, M., EMBO J., 1, 771-775, 1982); the bacteriophage lambda promoters and operators (Remaut, E. et al., Nucl. Acids Res., 11, 4677-4688, 1983); the α-amylase (B. subtilis) promoter and operator, termination sequences and other expression enhancement and control sequences compatible with the selected host cell.

When the host cell is yeast, useful expression control sequences include, e.g., α-mating factor. For insect cells the polyhedrin or p10 promoters of baculoviruses can be used (Smith, G.E. et al., Mol. Cell. Biol. 3, 2156-65, 1983). When the host cell is of vertebrate origin illustrative useful expression control sequences include the (human) cytomegalovirus immediate early promoter (Seed, B. et al., Nature 329, 840-842, 1987; Fynan, E.F. et al., PNAS 90, 11478-11482,1993; Ulmer, J.B. et al., Science 259, 1745-1748, 1993), Rous sarcoma virus LTR (RSV, Gorman, C.M. et al., PNAS 79, 6777-6781, 1982; Fynan et al., supra; Ulmer et al., supra), the MPSV LTR (Stacey et al., J. Virology 50, 725-732, 1984), SV40 immediate early promoter (Sprague J. et al., J. Virology 45, 773 ,1983), the SV-40 promoter (Berman, P.W. et al., Science, 222, 524-527, 1983), the metallothionein promoter (Brinster, R.L. et al., Nature 296, 39-42, 1982), the heat shock promoter (Voellmy et al., Proc. Natl. Acad. Sci. USA, 82, 4949-53, 1985), the major late promoter of Ad2 and the β-actin promoter (Tang et al., Nature 356, 152-154, 1992). The regulatory sequences may also include terminator and poly-adenylation sequences. Amongst the sequences that can be used are the well known bovine growth hormone poly-adenylation sequence, the SV40 poly-adenylation sequence, the human cytomegalovirus (hCMV) terminator and poly-adenylation sequences.

Bacterial, yeast, fungal, insect and vertebrate cell expression systems are very frequently used systems. Such systems are well-known in the art and generally available, e.g. commercially through Clontech Laboratories, Inc. 4030 Fabian Way, Palo Alto, California 94303-4607, USA. Next to these expression systems, parasite-based expression systems are attractive

expression systems. Such systems are e.g. described in the French Patent Application with Publication number 2 714 074, and in US NTIS Publication No US 08/043109 (Hoffman, S. and Rogers, W.: Public. Date 1 December 1993).

An even more preferred form of this embodiment of the invention relates to Live Recombinant Carriers (LRCs) comprising a nucleic acid encoding an *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof according to the invention, a DNA fragment according to the invention or a recombinant DNA molecule according to the invention. These LRCs are micro-organisms or viruses in which additional genetic information, in this case a nucleic acid encoding an *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, a DNA fragment or a recombinant DNA molecule according to the invention has been cloned. Chickens infected with such LRCs will produce an immunological response not only against the immunogens of the carrier, but also against the immunogenic parts of the protein(s) for which the genetic code is additionally cloned into the LRC, e.g. an *Ornithobacterium rhinotracheale* protein gene according to the invention.

As an example of bacterial LRCs, attenuated Salmonella strains known in the art can very attractively be used.
Also, live recombinant carrier parasites have i.a. been described by Vermeulen, A. N. (Int. Journ. Parasitol. 28: 1121-1130 (1998)).
Furthermore, LRC viruses may be used as a way of transporting the nucleic acid into a target cell. Live recombinant carrier viruses are also called vector viruses. Viruses often used as vectors are Vaccinia viruses (Panicali et al; Proc. Natl. Acad. Sci. USA, 79: 4927 (1982), Herpesviruses (E.P.A. 0473210A2), and Retroviruses (Valerio, D. et al; in Baum, S.J., Dicke, K.A., Lotzova, E. and Pluznik, D.H. (Eds.), Experimental Haematology today - 1988. Springer Verlag, New York: pp. 92-99 (1989)).
Viruses known and used in the art as very suitable vector viruses specifically in poultry are Fowlpox virus, Marek's serotype 3 virus, Herpes virus of Turkey, Semliki Forest virus and Newcastle Disease virus.

Live Recombinant Carriers are also known in the art as "live vectors", or shortly "vectors". Vaccines based upon a Live Recombinant Carrier are therefore also known in the art as vector vaccines.

The technique of *in vivo* homologous recombination, well-known in the art, can be used to introduce a recombinant nucleic acid into the genome of a bacterium, parasite or virus of

choice, capable of inducing expression of the inserted nucleic acid according to the invention in the host animal.

Finally another form of this embodiment of the invention relates to a host cell comprising a nucleic acid encoding a protein according to the invention, a DNA fragment comprising such a nucleic acid or a recombinant DNA molecule comprising such a nucleic acid under the control of a functionally linked promoter. This form also relates to a host cell containing a live recombinant carrier comprising a nucleic acid molecule encoding an *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof according to the invention. A host cell may be a cell of bacterial origin, e.g. *Escherichia coli*, *Bacillus subtilis* and *Lactobacillus* species, in combination with bacteria-based plasmids as pBR322, or bacterial expression vectors as pGEX, or with bacteriophages. The host cell may also be of eukaryotic origin, e.g. yeast-cells in combination with yeast-specific vector molecules, or higher eukaryotic cells like insect cells (Luckow et al; Bio-technology 6: 47-55 (1988)) in combination with vectors or recombinant baculoviruses, plant cells in combination with e.g. Ti-plasmid based vectors or plant viral vectors (Barton, K.A. et al; Cell 32: 1033 (1983), mammalian cells like Hela cells, Chinese Hamster Ovary cells (CHO) or Crandell Feline Kidney-cells, also with appropriate vectors or recombinant viruses.

Another embodiment of the invention relates to an *Ornithobacterium rhinotracheale* protein and to immunogenic fragments thereof according to the invention.

The concept of immunogenic fragments will be defined below.

One form of this embodiment relates to a 59.8 kD *Ornithobacterium rhinotracheale* protein and to immunogenic fragments thereof, having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 2.

In a preferred form, the embodiment relates to such *Ornithobacterium rhinotracheale* proteins and immunogenic fragments thereof, that have a sequence homology of at least 85 %, preferably 90 %, more preferably 95 % homology to the amino acid sequence as depicted in SEQ ID NO: 2.
Even more preferred is a homology level of 98 %, 99 % or even 100 %.

The level of protein homology can be determined with the computer program "BLAST 2 SEQUENCES" by selecting sub-program: "BLASTP", that can be found at **www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html.**

A reference for this program is Tatiana A. Tatusova, Thomas L. Madden FEMS Microbiol. Letters 174: 247-250 (1999). Matrix used: "blosum62". Parameters used are the default parameters:
Open gap: 11. Extension gap: 1. Gap x_dropoff: 50.

Another form of this embodiment relates to a 58.2 kD *Ornithobacterium rhinotracheale* protein and to immunogenic fragments thereof, having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 4.

In a preferred form, the embodiment relates to such *Ornithobacterium rhinotracheale* proteins and immunogenic fragments thereof, that have a sequence homology of at least 85 %, preferably 90 %, more preferably 95 % homology to the amino acid sequence as depicted in SEQ ID NO: 4.
Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Still another form of this embodiment relates to a 46.0 kD *Ornithobacterium rhinotracheale* protein and to immunogenic fragments thereof, having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 6.

In a preferred form, the embodiment relates to such *Ornithobacterium rhinotracheale* proteins and immunogenic fragments thereof, that have a sequence homology of at least 85 %, preferably 90 %, more preferably 95 % homology to the amino acid sequence as depicted in SEQ ID NO: 6.
Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Again another form of this embodiment relates to a 37.2 kD *Ornithobacterium rhinotracheale* protein and to immunogenic fragments thereof, having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 8.

In a preferred form, the embodiment relates to such *Ornithobacterium rhinotracheale* proteins and immunogenic fragments thereof, that have a sequence homology of at least 85 %, preferably 90 %, more preferably 95 % homology to the amino acid sequence as depicted in SEQ ID NO: 8.
Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Still another form of this embodiment relates to a 45.6 kD *Ornithobacterium rhinotracheale* protein and to immunogenic fragments thereof, having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 10.

In a preferred form, the embodiment relates to such *Ornithobacterium rhinotracheale* proteins and immunogenic fragments thereof, that have a sequence homology of at least 85 %, preferably 90 %, more preferably 95 % homology to the amino acid sequence as depicted in SEQ ID NO: 10.
Even more preferred is a homology level of 98 %, 99 % or even 100 %.

One other form of this embodiment relates to a 42.2 kD *Ornithobacterium rhinotracheale* protein and to immunogenic fragments thereof, having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 12.

In a preferred form, the embodiment relates to such *Ornithobacterium rhinotracheale* proteins and immunogenic fragments thereof, that have a sequence homology of at least 85 %, preferably 90 %, more preferably 95 % homology to the amino acid sequence as depicted in SEQ ID NO: 12.
Even more preferred is a homology level of 98 %, 99 % or even 100 %.

And again another form of this embodiment relates to a 34.0 kD *Ornithobacterium rhinotracheale* protein and to immunogenic fragments thereof, having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 14.

In a preferred form, the embodiment relates to such *Ornithobacterium rhinotracheale* proteins and immunogenic fragments thereof, that have a sequence homology of at least 85 %, preferably 90 %, more preferably 95 % homology to the amino acid sequence as depicted in SEQ ID NO: 14.
Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Finally another form of this embodiment relates to a 32.9 kD *Ornithobacterium rhinotracheale* protein and to immunogenic fragments thereof, having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO:

16.

In a preferred form, the embodiment relates to such *Ornithobacterium rhinotracheale* proteins and immunogenic fragments thereof, that have a sequence homology of at least 85 %, preferably 90 %, more preferably 95 % homology to the amino acid sequence as depicted in SEQ ID NO: 16.
Even more preferred is a homology level of 98 %, 99 % or even 100 %.

Another form of this embodiment relates to such *Ornithobacterium rhinotracheale* proteins and immunogenic fragments of said proteins according to the invention, wherein the proteins and immunogenic fragments thereof are encoded by a nucleic acid according to the invention.

It will be understood that, for the particular proteins embraced herein, natural variations can exist between individual *Ornithobacterium rhinotracheale* strains. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions which do not essentially alter biological and immunological activities, have been described, e.g. by Neurath et al in "The Proteins" Academic Press New York (1979). Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia, Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val (see Dayhof, M.D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3). Other amino acid substitutions include Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Thr/Phe, Ala/Pro, Lys/Arg, Leu/Ile, Leu/Val and Ala/Glu. Based on this information, Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science,227, 1435-1441, 1985) and determining the functional similarity between homologous proteins. Such amino acid substitutions of the exemplary embodiments of this invention, as well as variations having deletions and/or insertions are within the scope of the invention as long as the resulting proteins retain their immune reactivity.
This explains why *Ornithobacterium rhinotracheale* proteins according to the invention, when isolated from different field isolates, may have homology levels as low as about 80%, while still representing the same protein with the same immunological characteristics. Those variations in the amino acid sequence of a certain protein according to the invention that still provide a protein capable of inducing an immune response against infection with *Ornithobacterium rhinotracheale* or at least against the clinical manifestations of the infection are considered as "not essentially influencing the immunogenicity".

When a protein is used for e.g. vaccination purposes or for raising antibodies, it is however not necessary to use the whole protein. It is also possible to use a fragment of that protein that is capable, as such or coupled to a carrier such as e.g. KLH, of inducing an immune response against that protein, a so-called immunogenic fragment. An "immunogenic fragment" is understood to be a fragment of the full-length protein that still has retained its capability to induce an immune response in a vertebrate host, e.g. comprises a B- or T-cell epitope. Shortly, an immunogenic fragment is a fragment that is capable of inducing an antigenic response against an *Ornithobacterium rhinotracheale* protein according to the invention. At this moment, a variety of techniques is available to easily identify DNA fragments encoding antigenic fragments (determinants). The method described by Geysen et al (Patent Application WO 84/03564, Patent Application WO 86/06487, US Patent NR. 4,833,092, Proc. Natl Acad. Sci. 81: 3998-4002 (1984), J. Imm. Meth. 102, 259-274 (1987), the so-called PEPSCAN method is an easy to perform, quick and well-established method for the detection of epitopes; the immunologically important regions of the protein. The method is used world-wide and as such well-known to man skilled in the art. This (empirical) method is especially suitable for the detection of B-cell epitopes. Also, given the sequence of the gene encoding any protein, computer algorithms are able to designate specific protein fragments as the immunologically important epitopes on the basis of their sequential and/or structural agreement with epitopes that are now known. The determination of these regions is based on a combination of the hydrophilicity criteria according to Hopp and Woods (Proc. Natl. Acad. Sci. 78: 38248-3828 (1981)), and the secondary structure aspects according to Chou and Fasman (Advances in Enzymology 47: 45-148 (1987) and US Patent 4,554,101). T-cell epitopes can likewise be predicted from the sequence by computer with the aid of Berzofsky's amphiphilicity criterion (Science 235, 1059-1062 (1987) and US Patent application NTIS US 07/005,885). A condensed overview is found in: Shan Lu on common principles: Tibtech 9: 238-242 (1991), Good et al on Malaria epitopes; Science 235: 1059-1062 (1987), Lu for a review; Vaccine 10: 3-7 (1992), Berzofsky for HIV-epitopes; The FASEB Journal 5:2412-2418 (1991). An immunogenic fragment usually has a minimal length of 8 amino acids, preferably more then 8, such as 9, 10, 12, 15 or even 20 amino acids. The nucleic acids encoding such a fragment therefore have a length of at least 24, but preferably 27, 30, 36, 45 or even 60 nucleic acids.

Therefore, one form of still another embodiment of the invention relates to vaccines for combating *Ornithobacterium rhinotracheale* infection, that comprise an *Ornithobacterium rhinotracheale* protein or immunogenic fragments thereof, according to the invention as described above together with a pharmaceutically acceptable carrier.

Still another embodiment of the present invention relates to an *Ornithobacterium rhinotracheale* protein according to the invention or immunogenic fragments thereof for use in a vaccine.

Still another embodiment of the present invention relates to the use of a nucleic acid, a DNA fragment, a recombinant DNA molecule, a live recombinant carrier, a host cell or a protein or an immunogenic fragment thereof according to the invention for the manufacturing of a vaccine for combating *Ornithobacterium rhinotracheale* infection.

One way of making a vaccine according to the invention is by growing the bacteria, followed by biochemical purification of an *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, from the bacterium. This is however a very time-consuming way of making the vaccine.

It is therefore much more convenient to use the expression products of the gene encoding an *Ornithobacterium rhinotracheale* protein or immunogenic fragments thereof in vaccines. This is possible for the first time now because the nucleic acids encoding the *Ornithobacterium rhinotracheale* proteins are provided in the present invention.

Vaccines based upon the expression products of these genes can easily be made by admixing the protein according to the invention or immunogenic fragments thereof according to the invention with a pharmaceutically acceptable carrier as described below.

Alternatively, a vaccine according to the invention can comprise live recombinant carriers as described above, capable of expressing the protein according to the invention or immunogenic fragments thereof. Such vaccines, e.g. based upon a *Salmonella* carrier or a viral carrier e.g. a Herpesvirus vector have the advantage over subunit vaccines that they better mimic the natural way of infection of *Ornithobacterium rhinotracheale*. Moreover, their self-propagation is an advantage since only low amounts of the recombinant carrier are necessary for immunization.

Vaccines can also be based upon host cells as described above, that comprise the protein or immunogenic fragments thereof according to the invention.

All vaccines described above contribute to active vaccination, i.e. they trigger the host's defense system.

Alternatively, antibodies can be raised in e.g. rabbits or can be obtained from antibody-producing cell lines as described below. Such antibodies can then be administered to the chicken. This method of vaccination, passive vaccination, is the vaccination of choice when an animal is already infected, and there is no time to allow the natural immune response to be triggered. It is also the preferred method for vaccinating animals that are prone to sudden high infection pressure. The administered antibodies against the protein according to the invention or immunogenic fragments thereof can in these cases bind directly to *Ornithobacterium rhinotracheale*. This has the advantage that it decreases or stops *Ornithobacterium rhinotracheale* multiplication.

Therefore, one other form of this embodiment of the invention relates to a vaccine for combating *Ornithobacterium rhinotracheale* infection that comprises antibodies against a *Ornithobacterium rhinotracheale* protein according to the invention or an immunogenic fragment of that protein, and a pharmaceutically acceptable carrier.

Still another embodiment of this invention relates to antibodies against a *Ornithobacterium rhinotracheale* protein according to the invention or an immunogenic fragment of that protein.

Methods for large-scale production of antibodies according to the invention are also known in the art. Such methods rely on the cloning of (fragments of) the genetic information encoding the protein according to the invention in a filamentous phage for phage display. Such techniques are described i.a. at the "Antibody Engineering Page" under "filamentous phage display" at http://aximt1.imt.uni-marburg.de/~rek/aepphage.html., and in review papers by Cortese, R. et al., (1994) in Trends Biotechn. 12: 262-267., by Clackson, T. & Wells, J.A. (1994) in Trends Biotechn. 12: 173-183, by Marks, J.D. et al., (1992) in J. Biol. Chem. 267: 16007-16010, by Winter, G. et al., (1994) in Annu. Rev. Immunol. 12: 433-455, and by Little, M. et al., (1994) Biotechn. Adv. 12: 539-555. The phages are subsequently used to screen camelid expression libraries expressing camelid heavy chain antibodies. (Muyldermans, S. and Lauwereys, M., Journ. Molec. Recogn. 12: 131-140 (1999) and Ghahroudi, M.A. et al., FEBS Letters 414: 512-526 (1997)). Cells from the library that express the desired antibodies can be replicated and subsequently be used for large scale expression of antibodies.

Still another embodiment relates to a method for the preparation of a vaccine according to the invention that comprises the admixing of antibodies according to the invention and a pharmaceutically acceptable carrier.

An alternative and efficient way of vaccination is direct vaccination with DNA encoding the relevant antigen. Direct vaccination with DNA encoding proteins has been successful for many different proteins. (As reviewed in e.g. Donnelly et al., The Immunologist 2: 20-26 (1993)). This way of vaccination is also attractive for the vaccination of chickens against *Ornithobacterium rhinotracheale* infection.

Therefore, still other forms of this embodiment of the invention relate to vaccines comprising nucleic acids encoding a protein according to the invention or immunogenic fragments thereof, comprising DNA fragments that comprise such nucleic acids or comprising recombinant DNA molecules according to the invention, and a pharmaceutically acceptable carrier.

Examples of DNA plasmids that are suitable for use in a DNA vaccine according to the invention are conventional cloning or expression plasmids for bacterial, eukaryotic and yeast host cells, many of said plasmids being commercially available. Well-known examples of such plasmids are pBR322 and pcDNA3 (Invitrogen). The DNA fragments or recombinant DNA molecules according to the invention should be able to induce protein expression of the nucleotide sequences. The DNA fragments or recombinant DNA molecules may comprise one or more nucleotide sequences according to the invention. In addition, the DNA fragments or recombinant DNA molecules may comprise other nucleotide sequences such as the immune-stimulating oligonucleotides having unmethylated CpG di-nucleotides, or nucleotide sequences that code for other antigenic proteins or adjuvating cytokines.

The nucleotide sequence according to the present invention or the DNA plasmid comprising a nucleotide sequence according to the present invention, preferably operably linked to a transcriptional regulatory sequence, to be used in the vaccine according to the invention can be naked or can be packaged in a delivery system. Suitable delivery systems are lipid vesicles, iscoms, dendromers, niosomes, polysaccharide matrices and the like, (see further below) all well-known in the art. Also very suitable as delivery system are attenuated live bacteria such as Salmonella species, and attenuated live viruses such as Herpesvirus vectors, as mentioned above.

DNA vaccines can e.g. easily be administered through intradermal application such as by using a needle-less injector. This way of administration delivers the DNA directly into the cells of the animal to be vaccinated. Amounts of DNA in the range between 10 pg and 1000 µg provide good results. Preferably, amounts in the microgram range between 1 and 100 µg are used.

In a further embodiment, the vaccine according to the present invention comprises one or more additional antigens derived from a virus or micro-organism pathogenic to poultry, an antibody against such an antigen or genetic information encoding said antigen.

Of course, such antigens can be e.g. other *Ornithobacterium rhinotracheale* antigens. It is beneficial to combine, in one vaccine, two or more of the proteins or immunogenic fragments thereof according to the invention, antibodies against such proteins or immunogenic fragments thereof, or genetic information encoding such proteins or immunogenic fragments thereof.

Next to this, it is beneficial to include in a vaccine according to the invention, antigens derived from another micro-organism or a virus pathogenic to poultry, an antibody against such an antigen or genetic information encoding said antigen.

Preferably, the virus or micro-organism is selected from the group consisting of Fowlpox virus, Infectious Bronchitis virus, Infectious Bursal Disease (Gumboro), Marek's Disease Virus, Chicken Anaemia agent, Avian Reovirus, *Mycoplasma gallisepticum*, Turkey Rhinotracheitis virus, *Haemophilus paragallinarum* (Coryza), Chicken Poxvirus, Avian Encephalomyelitisvirus, Duck Plague virus, Newcastle Disease virus, Egg Drop syndrome virus, Infectious Laryngotracheitis virus, Herpes Virus of Turkeys, Eimeria species, *Ornithobacterium rhinotracheale, Pasteurella multocida, Mycoplasma synoviae, Salmonella* species and *E. coli.*

Vaccines based upon the *Ornithobacterium rhinotracheale* proteins according to the invention are also very suitable as marker vaccines. A marker vaccine is a vaccine that allows to discriminate between vaccinated and field-infected chickens e.g. on the basis of a characteristic antibody panel, different from the antibody panel induced by wild type infection. A different antibody panel is induced e.g. when an immunogenic protein present on a wild type bacterium is not present in a vaccine: the host will then not make antibodies against that protein after vaccination. Thus, a vaccine based upon an *Ornithobacterium rhinotracheale* protein according to the invention would only induce antibodies against that protein, whereas a vaccine based upon a live wild-type, live attenuated or inactivated whole *Ornithobacterium rhinotracheale* would induce antibodies against all or most of the bacterial proteins.

A simple ELISA test, having wells comprising one protein according to the invention and wells comprising another protein according to the invention suffices to test serum from chickens and to tell if the chickens are either vaccinated with a subunit vaccine according to the invention or suffered from *Ornithobacterium rhinotracheale* field infection; chickens

vaccinated with a vaccine comprising one protein according to the invention would not have antibodies against another protein according to the invention. Chickens that have encountered a field infection with *Ornithobacterium rhinotracheale* would however have antibodies against all immunogenic *Ornithobacterium rhinotracheale* proteins and thus also against another protein according to the invention.

All vaccines according to the present invention comprise a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier can be e.g. sterile water or a sterile physiological salt solution. In a more complex form the carrier can e.g. be a buffer.

Methods for the preparation of a vaccine comprise the admixing of a protein or an immunogenic fragment thereof, according to the invention and/or antibodies against that protein or an immunogenic fragment thereof, and/or a nucleic acid and/or a DNA fragment, a recombinant DNA molecule, a live recombinant carrier or host cell according to the invention, and a pharmaceutically acceptable carrier.

Vaccines according to the present invention may in a preferred presentation also contain an immunostimulatory substance, a so-called adjuvant. Adjuvants in general comprise substances that boost the immune response of the host in a non-specific manner. A number of different adjuvants are known in the art. Examples of adjuvants frequently used in chicken vaccines are muramyldipeptides, lipopolysaccharides, several glucans and glycans and Carbopol(R) (a homopolymer).
The vaccine may also comprise a so-called "vehicle". A vehicle is a compound to which the protein adheres, without being covalently bound to it. Such vehicles are i.a. bio-microcapsules, micro-alginates, liposomes and macrosols, all known in the art.
A special form of such a vehicle, in which the antigen is partially embedded in the vehicle, is the so-called ISCOM (EP 109.942, EP 180.564, EP 242.380)
In addition, the vaccine may comprise one or more suitable surface-active compounds or emulsifiers, e.g. Span or Tween.

Often, the vaccine is mixed with stabilisers, e.g. to protect degradation-prone proteins from being degraded, to enhance the shelf-life of the vaccine, or to improve freeze-drying efficiency. Useful stabilisers are i.a. SPGA (Bovarnik et al; J. Bacteriology 59: 509 (1950)), carbohydrates e.g. sorbitol, mannitol, trehalose, starch, sucrose, dextran or glucose, proteins such as albumin or casein or degradation products thereof, and buffers, such as alkali metal phosphates.

In addition, the vaccine may be suspended in a physiologically acceptable diluent.
It goes without saying, that other ways of adjuvating, adding vehicle compounds or diluents, emulsifying or stabilising a protein are also embodied in the present invention.

Vaccines according to the invention that are based upon the protein according to the invention or immunogenic fragments thereof can very suitably be administered in amounts ranging between 1 and 100 micrograms of protein per animal, although smaller doses can in principle be used. A dose exceeding 100 micrograms will, although immunologically very suitable, be less attractive for commercial reasons.

Vaccines based upon live attenuated recombinant carriers, such as the LRC-viruses and bacteria described above can be administered in much lower doses, because they multiply themselves during the infection. Therefore, very suitable amounts would range between 10^3 and 10^9 CFU/PFU for respectively bacteria/viruses.

Vaccines according to the invention can be administered e.g. intradermally, subcutaneously, intramuscularly, intraperitoneally, intravenously, or at mucosal surfaces such as orally or intranasally.
Live recombinant carrier vaccines or vector vaccines can most efficiently be administered by spraying, by aerosol or by drinking water administration.

Examples.

Example 1: Library construction, sera and screening.

For the construction of an expression library of *Ornithobacterium rhinotracheale* serotype G strain O-95029 nr.16279, genomic DNA was isolated from cells grown in Todd Hewitt broth (THB) for 24 hours at 37°C on a 100 rpm shaker, according to the method described in Maniatis/Sambrook (Sambrook, J. *et al.* Molecular cloning: a laboratory manual. ISBN 0-87969-309-6). DNA fragments of 1 – 4 kb were obtained by restriction enzyme digestion and ligated into λTriplEx vector arms (Clontech, Palo Alto, CA, USA). Subsequent packaging was performed using the Stratagene (La Jolla, CA, USA) *in vitro* packaging extract. *Escherichia coli* XL1 Blue cells, grown in Luria Bertani (LB) broth supplemented with 10 mM $MgSO_4$ and 0.2% maltose, were used for transfection. The complexity of the constructed expression library was tested 6.9 and it contained 97% recombinants.

The *Ornithobacterium rhinotracheale* serotype G expression library was screened with polyclonal antisera directed against whole live organisms of several *Ornithobacterium rhinotracheale* serotypes. Sera were collected from broiler chickens that were vaccinated by aerosol spraying with live *Ornithobacterium rhinotracheale* bacteria of serotype B (strain GGD 1261), serotype G (strain O-95029 nr.16279) or serotype M (strain TOP 98036 4500) at two weeks of age. Three weeks later the chickens were intravenously challenged with *Ornithobacterium rhinotracheale* serotype A (strain B3263/91). Sera were collected one week after challenge. All vaccinated birds showed reduced pathology (ranging from 10% to 60%) in comparison to unvaccinated control birds. Before use in expression library screening, the antisera were adsorbed with *Escherichia coli* XL1 Blue cell lysate as described in Maniatis/Sambrook (Sambrook, J. *et al.* Molecular cloning: a laboratory manual. ISBN 0-87969-309-6) in order to reduce a-specific background signal.

The expression library was screened by plaque lift using an initial screening of approximately 20.000 plaques. The procedure was done as described in the manufacturers handbook (Clontech, Palo Alto, CA, USA). All library screenings were done under native conditions. In short, phage-infected *Escherichia coli* XL1 Blue cells were plated in LB top agar onto LB agar plates both supplemented with 10 mM $MgSO_4$. The plates were then incubated at 42°C for 4 hours. A nitrocellulose filter disc (Schleicher and Schuell, Dassel, Germany), previously soaked in 10 mM IPTG, was placed on each plate in order to induce expression of the proteins encoded by the cloned *Ornithobacterium rhinotracheale* inserts. After 4 hours incubation at 37°C all filters were removed from the plates. After washing and blocking, filters were

incubated with chicken antiserum (pooled from 10 animals, 1:250 dilution). The antiserum used in the first screening was obtained from chickens live vaccinated with *Ornithobacterium rhinotracheale* serotype G followed by a challenge with *Ornithobacterium rhinotracheale* serotype A. As secondary antibody rabbit anti-chicken IgG peroxidase (Nordic, Tilburg, The Netherlands) was use at 1:1000 dilution. As substrate solution Vector SG (Vector, Burlingame, CA, USA) was used.

From the initial screening of 20.000 plaques, 200 reactive plaques were located on the agar plates and isolated. A plaque lift and screen as described above was repeated twice resulting in 175 single, pure reactive plaques. The pure clones were then spotted *in duplo* onto an *E.coli* XL1 Blue top agar lawn to give confluent plaques of approximately 5 mm diameter. Again a plaque lift was performed and the filters were incubated with the antisera obtained from birds live vaccinated with *Ornithobacterium rhinotracheale* serotype B or serotype M prior to *Ornithobacterium rhinotracheale* serotype A challenge. Out of 175 reactive plaques, 30 plaques were selected to be cross-reactive with sera from birds live vaccinated with *Ornithobacterium rhinotracheale* serotype B, serotype G, or serotype M, and challenged with *Ornithobacterium rhinotracheale* serotype A.

Example 2: Identification of open reading frames (ORFs) encoding antigenic proteins and expression in *Escherichia coli.*

The DNA inserts of the 30 selected plaques were analysed in order to identify the open reading frames encoding the antigenic proteins. Oligonucleotide primers designed for the λTriplEx vector arms were used for both PCR amplification and sequencing. PCR was performed in a final reaction volume of 50 µl containing 50 µM dNTP's (Promega, WI, USA), 10 pmol of both primers, 20 U/ml Supertaq plus polymerase and 10X Supertaq buffer (both HT Biotechnology Ltd, Cambridge, UK) in water. Phage DNA was added by picking a freshly plated plaque using a tooth pick, and transferring this DNA from tooth pick to reaction mix. The following conditions were used: denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min and elongation at 68°C for 2 min 30 sec, followed by a final extension at 68°C for 10 min. To determine the nucleotide sequence of the amplified DNA inserts a sequence reaction was done (94°C 10 sec; 50°C 5 sec; 60°C 2 min for 25 cycles) using Big dye Terminator Ready reaction mix (Qiagen Inc., CA, USA), 50 ng template DNA (PCR product) and 2.4 pmol primer in a 20µl reaction volume.

After sequence analysis the 30 clones appeared to represent 8 different genes. Since most open reading frames where a fusion with the lacZ gene of the λTriplEx vector, the 5'end of

the gene was missing. For that reason a sequence reaction was performed using internal primers and chromosomal DNA of *Ornithobacterium rhinotracheale* serotype G as a template to sequence the missing 5'gap.

Oligonucleotide primers were designed to amplify the full length open reading frames encoding the 8 cross-reactive antigens (Or01, Or02, Or03, Or04, Or11, Or77, Or98A and Or98B) from genomic DNA of *Ornithobacterium rhinotracheale* serotype G strain O-95029 nr.16279 (see table 1). The 5'oligonucleotide primers contain a restriction site (underlined) preceding the ATG initiation codon (bold) followed by sequences derived from the gene of interest (italic). The 3'oligonucleotides contain coding sequences (italic) followed by a restriction site (underlined). The PCR products were cloned in the expression vector of interest. Ligation products were transformed to *E.coli* BL21 (DE3) codon RIL pLysS host cells (Novagen, Madison, WI, USA) for protein expression. By using the pET plasmid vector (pET22b) and a T7 RNA polymerase expression system (Novagen, Madison, WI, USA), the recombinant proteins were expressed in *E.coli,* with an *E.coli pelB* leader peptide fused at the amino terminal portion (*Ornithobacterium rhinotracheale* leader peptides of proteins Or02, Or03, Or11, and Or77 were replaced) and 6 histidine residues at the carboxy terminal portion of the protein. *E.coli* strain BL21 (DE3) codon RIL pLysS (Novagen, Madison, WI, USA) was used for high level expression during IPTG-induction as described in the pET system manual (Novagen, Madison, WI, USA).

Example 3: Purification of antigens, vaccine formulations and serological analysis.

Recombinant antigens expressed in *E.coli* were isolated from supernatant (Or77), purified by metal affinity chromatography using talon resin (Clontech Inc., Palo Alto, CA, USA) as described by the manufacturer (Or03, Or04, Or98A and Or98B), or by repeated freeze-thawing, sonification, and centrifugation cycli (Or01, Or02 and Or11). Polyacrylamide gel electrophoresis (PAGE) followed by Coomassie brilliant blue staining was used to assess the purity of the recombinant proteins. Protein concentrations were estimated using bovine serum albumin as the standard.

All purified recombinant proteins (Or01, Or02, Or03, Or04, Or11, Or77, Or98A and Or98B) were formulated individually in a water in oil emulsion. Furthermore, five different subunit vaccines (A, B, C, D and E) were formulated, containing different compositions of the 8 recombinant antigens (table 2). Coomassie staining of the 5 combination vaccines showed clearly identifiable protein bands corresponding to recombinant proteins Or01, Or02 and

Or77. As the molecular weights of Or03, Or04 and Or11, and the molecular weights of Or98A and Or98B are approximately the same, individual protein bands could not be distinguished (figure 1). All proteins are present in approximately equal concentrations of 50 mg/antigen/l (25 µg/dose). Therefore, the total antigenic load of vaccine A to D is 200 mg/l. The antigen concentration of vaccine E is 400 mg/l. The protein background is rest material from *E.coli* strain used to express the recombinant *Ornithobacterium rhinotracheale* antigens.

The ability of the different subunit vaccines to stimulate the humoral immune response to produce protein-specific antibodies was studied by subcutaneous injection of 2-weeks-old SPF-broiler chickens with 0.5 ml vaccine. Four weeks after vaccination serum-samples were collected and tested for the presence of antibodies reactive against the recombinant proteins. Semi-dry Western blotting was performed according to Towbin, H., Staehlin, T., and Gordon, J. (1979) Proc. Nat. Acad. Sci. 76:43-50. The protein phase of the vaccines was blotted and incubated with pooled serum (1:100 dilution) from vaccinated and unvaccinated birds. Sera obtained from birds vaccinated with each of the 8 individual vaccines Or01 to Or98B showed protein-specific reactivity (figure 2). Figure 3 shows the reactivity of antisera obtained from birds vaccinated with subunit vaccine A to E (see table 2 and figure 1), directed against the same vaccines on Western blot. For example: blot A is loaded with vaccine A, B, C, D, and E (corresponding with lanes A to E). The serum used for primary antibody binding is obtained from birds vaccinated with vaccine A (corresponds with blot-number). For this reason, α-Or01, α-Or02, α-Or03 and α-Or04 antibodies are present in this serum. On blot A, these four proteins are stained in lane A, D, and E, which are the lanes that were loaded with the three vaccines that contain these antigens (A, D, and E). Blot B is loaded as blot A and the serum used is obtained from birds vaccinated with vaccine B. α-Or77, α-Or11, α-Or03, and α-Or04 antibodies stain the corresponding antigens on blot B in lane B, C, and E. The other antigens that were not present in vaccine B could not be detected on this blot. On blot E, all proteins are stained because vaccine E contains all eight *Ornithobacterium rhinotracheale* antigens. The serum used on Westernblot F is obtained from unvaccinated birds that served as a negative control. No recombinant *Ornithobacterium rhinotracheale* antigens could be detected using this serum.

Example 4: Protection studies.

To assess the cross-protective capacity of the antibody response induced by different subunit vaccines (combi vaccines A, B, C, D, E, and individual vaccine Or77), an animal experiment was performed. SPF-broilers were vaccinated at 2 weeks of age as described before. At 5

weeks of age birds were primed with ND LaSota (dose:$1*10^6$ E.I.D.per bird) by aerosol spraying. At 6 weeks of age, birds were challenged with *Ornithobacterium rhinotracheale* serotype A strain B3263/91 (heterologous challenge). The challenge was done by aerosol spraying of a fresh bacterial culture containing $8.5*10^8$ colony forming units (CFU) per ml THB. During aerosol challenge the bacterial culture was administered as a fine spray to the birds in an isolator of approximately $1.5m^3$, using a commercial paint sprayer. The developed mist in the isolators was maintained for at least 10 min with the air circulation closed. Challenge control groups and ND priming groups were included in the test. One week after challenge, at 7 weeks of age, birds were sacrificed and organ lesions were macroscopically scored using an *Ornithobacterium rhinotracheale* scoring system for respiratory disease as follows: for thoracic air sacs, 0= no abnormalities, 1= one air sac seriously affected by fibrinous airsacculitis or limited pin-head sized foci of fibrinous exudates in both air sacs, 2= both air sacs seriously affected by fibrinous airsacculitis; for abdominal air sacs, 0= no abnormalities, 1= pin-head sized foci of fibrinous exudates or slight diffuse fibrinous airsacculitis, 2= severe fibrinous airsacculitis. The airsacculitis score is given as the sum of both scores. For lungs, 0= no abnormalities, 1= unilateral pneumonia, 2= bilateral pneumonia. The average group scores are given as a percentage of the maximum possible score. Statistical analysis was performed using Kruskal-Wallis non-parametric one-way ANOVA.

Figure 4 shows the cross-protective capacity of the 5 different subunit vaccines A to E. The challenge control group was not vaccinated but primed and challenged and showed the highest score. Birds vaccinated with vaccine E (containing all 8 antigens) showed almost complete protection comparable to the results of the group that did not receive vaccination and challenge but was primed with Newcastle Disease virus. A somewhat lesser, but still significant cross-protection ($P<0.05$) could be observed in birds vaccinated with vaccine A, B and C. Combination vaccine D showed cross-protection of less significance ($p=0.19$). Untreated birds showed no organ lesions.

As can be seen from figure 5, the Or77 (= serotype G strain)-vaccinated and serotype A challenged animals also show a significant ($p<0.05$)) reduction in respiratory lesion scores compared to the unvaccinated control group.

Legend to the figures:

Figure 1: Coomassie staining of the 5 combination vaccines (A to E). Each vaccine containing a different composition of the 8 purified recombinant proteins. Subunit vaccine A corresponds with lane A, subunit vaccine B corresponds with lane B, subunit vaccine C corresponds with lane C, subunit vaccine D corresponds with lane D, subunit vaccine E corresponds with lane E. Recombinant proteins with approximately equal molecular weights are indicated by a single arrow.

Figure 2: Reactivity of monovalent antisera, obtained from chickens vaccinated with the single recombinant subunit vaccines, against the same protein on Western blot. The reactive vaccine proteins are indicated with black arrows.

Figure 3: Reactivity of antisera, obtained from chickens vaccinated with subunit vaccines A to E on Western blot. Each blot contains the proteins of vaccine A, B, C, D, and E (corresponding to lanes A to E). The serum used for screening is obtained from birds vaccinated with vaccine A (blot A), vaccine B (blot B), vaccine C (blot C), vaccine D (blot D) or vaccine E (blot E). The serum used on Western blot F is obtained from unvaccinated birds. The reactive vaccine proteins are indicated with a black line.

Figure 4: Cross-protective capacity of subunit vaccines A to E, in comparison to challenge and NDV control groups, represented as the maximum possible respiratory organ lesion score.

Figure 5: Cross-protective capacity of subunit vaccine Or77, in comparison to challenge and NDV control groups, represented as the maximum possible respiratory organ lesion score.

Gene	5'oligonucleotide	Restriction site	3'oligonucleotide	Restriction site
Or01	5'-GCTGGCC**ATG***GCTGAAATTATAAAAATGCC*-3'	MscI	5'-CCGCTCGAG*CACAAGCATAGACATTGG*-3'	XhoI
Or02	5'-CAGTCC**ATGG***CATGTAGCGATTTTGAT*-3'	NcoI	5'-CCGCTCGAG*GTGGTCTTTATAAAAATG*-3'	XhoI
Or03	5'-CAGTCC**ATGG***CGATGATAATCAGTTCTTATG*-3'	NcoI	5'-CCGCTCGAG*AATAAATTCATCATTAAGC*-3'	XhoI
Or04	5'-CGATGGCC**ATG***AAAGATATATTTGAAT*-3'	MscI	5'-CCGCTCGAG*TTCTTCACTTGGTATTTTGA*-3'	XhoI
Or11	5'-CGATGGCC**ATG***GGGGCACAAGGTGTAGC*-3'	MscI	5'-GCGGCCGC*TACGATAAACCTAGACCAAA*-3'	NotI
Or77	5'-CATGCC**ATGG***TCTGTAGCAGTGATGATTAC*-3'	NcoI	5'-CCGCTCGAG*GTTAATTGAAACTCTTAAGC*-3'	XhoI
Or98A	5'-CAGTCC**ATGG***TAAAAGACTTTTCAG*-3'	NcoI	5'-CCGCTCGAG*TGCTATTAATTCTAATCG*-3'	XhoI
Or98B	5'-CAGTCC**ATGG***AATTAGCGAAAAACGAC*-3'	NcoI	5'-CCGCTCGAG*TTTTAATTCATTTTTTCTG*-3'	XhoI

Restriction site: underlined

ATG start codon: **bold**

Gene of interest: *italic*

Table 1: Oligonucleotide sets used for cloning selected *Ornithobacterium rhinotracheale* genes encoding cross-reactive antigens

Vaccine	Antigen							
	Or01	**Or02**	**Or03**	**Or04**	**Or11**	**Or77**	**Or98A**	**Or98B**
A								
B								
C								
D								
E								

: antigen is present in the vaccine

Table 2: Subunit vaccines (A to E) consisting of different protein subset combinations

Claims

1) Nucleic acid encoding a 59.8 kD *Ornithobacterium rhinotracheale* protein or a part of said nucleic acid that encodes an immunogenic fragment of said protein, said nucleic acid or said part thereof having at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 1.
2) Nucleic acid or part thereof according to claim 1, characterized in that the sequence has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 1.
3) Nucleic acid encoding a 58.2 kD *Ornithobacterium rhinotracheale* protein or a part of said nucleic acid that encodes an immunogenic fragment of said protein, said nucleic acid or said part thereof having at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 3.
4) Nucleic acid or part thereof according to claim 3, characterized in that the sequence has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 3.
5) Nucleic acid encoding a 46.0 kD *Ornithobacterium rhinotracheale* protein or a part of said nucleic acid that encodes an immunogenic fragment of said protein, said nucleic acid or said part thereof having at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 5.
6) Nucleic acid or part thereof according to claim 5, characterized in that the sequence has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 5.
7) Nucleic acid encoding a 37.2 kD *Ornithobacterium rhinotracheale* protein or a part of said nucleic acid that encodes an immunogenic fragment of said protein, said nucleic acid or said part thereof having at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 7.
8) Nucleic acid or part thereof according to claim 7, characterized in that the sequence has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 7.
9) Nucleic acid encoding a 45.6 kD *Ornithobacterium rhinotracheale* protein or a part of said nucleic acid that encodes an immunogenic fragment of said protein, said

nucleic acid or said part thereof having at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 9.

10) Nucleic acid or part thereof according to claim 9, characterized in that the sequence has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 9.

11) Nucleic acid encoding a 42.2 kD *Ornithobacterium rhinotracheale* protein or a part of said nucleic acid that encodes an immunogenic fragment of said protein, said nucleic acid or said part thereof having at least 80 % homology with the nucleic acid of he *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 11.

12) Nucleic acid or part thereof according to claim 11, characterized in that the sequence has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 11.

13) Nucleic acid encoding a 34.0 kD *Ornithobacterium rhinotracheale* protein or a part of said nucleic acid that encodes an immunogenic fragment of said protein, said nucleic acid or said part thereof having at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 13.

14) Nucleic acid or part thereof according to claim 13, characterized in that the sequence has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 13.

15) Nucleic acid encoding a 32.9 kD *Ornithobacterium rhinotracheale* protein or a part of said nucleic acid that encodes an immunogenic fragment of said protein, said nucleic acid or said part thereof having at least 80 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 15.

16) Nucleic acid or part thereof according to claim 15, characterized in that the sequence has at least 85 %, preferably 90 %, more preferably 95 % homology with the nucleic acid of the *Ornithobacterium rhinotracheale* protein gene as depicted in SEQ ID NO: 15.

17) DNA fragment comprising a nucleic acid according to claim 1-16.

18) Recombinant DNA molecule comprising a nucleic acid according to claims 1-16 or a DNA fragment according to claim 17, under the control of a functionally linked promoter.

19) Live recombinant carrier comprising a nucleic acid according to claims 1-16, a DNA fragment according to claim 17 or a recombinant DNA molecule according to claim 18.

20) Host cell comprising a nucleic acid according to claims 1-16, a DNA fragment according to claim 17, a recombinant DNA molecule according to claim 18 or a live recombinant carrier according to claim 19.

21) A 59.8 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 2.

22) A *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, according to claim 21, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 85 %, preferably 90 %, more preferably 95 % to the amino acid sequence as depicted in SEQ ID NO: 2.

23) A 59.8 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, characterized in that it is encoded by a nucleic acid according to claim 1 or 2.

24) A 58.2 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 80 % to the amino acid sequence as depicted in SEQ ID NO: 4.

25) A *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, according to claim 24, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 85 %, preferably 90 %, more preferably 95 % to the amino acid sequence as depicted in SEQ ID NO: 4.

26) A 58.2 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, characterized in that it is encoded by a nucleic acid according to claim 3 or 4.

27) A 46.0 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 6.

28) A *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, according to claim 27, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 85 %, preferably 90 %, more preferably 95 % to the amino acid sequence as depicted in SEQ ID NO: 6.

29) A 46.0 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, characterized in that it is encoded by a nucleic acid according to claim 5 or 6.

30) A 37.2 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 8.

31) A *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, according to claim 30, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 85 %, preferably 90 %, more preferably 95 % to the amino acid sequence as depicted in SEQ ID NO: 8.

32) A 37.2 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, characterized in that it is encoded by a nucleic acid according to claim 7 or 8.

33) A 45.6 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 10.

34) A *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, according to claim 33, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 85 %, preferably 90 %, more preferably 95 % to the amino acid sequence as depicted in SEQ ID NO: 10.

35) A 45.6 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, characterized in that it is encoded by a nucleic acid according to claim 9 or 10.

36) A 42.2 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 12.

37) A *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, according to claim 36, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 85 %, preferably 90 %, more preferably 95 % to the amino acid sequence as depicted in SEQ ID NO: 12.

38) A 42.2 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, characterized in that it is encoded by a nucleic acid according to claim 11 or 12.

39) A 34.0 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 14.

40) A *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, according to claim 39, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 85 %, preferably 90 %, more preferably 95 % to the amino acid sequence as depicted in SEQ ID NO: 14.

41) A 34.0 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, characterized in that it is encoded by a nucleic acid according to claim 13 or 14.

42) A 32.9 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 80 % with the amino acid sequence as depicted in SEQ ID NO: 16.

43) A *Ornithobacterium rhinotracheale* protein or an immunogenic fragment of said protein, according to claim 42, said protein or immunogenic fragment thereof having an amino acid sequence homology of at least 85 %, preferably 90 %, more preferably 95 % to the amino acid sequence as depicted in SEQ ID NO: 16.

44) A 32.9 kD *Ornithobacterium rhinotracheale* protein or an immunogenic fragment thereof, characterized in that it is encoded by a nucleic acid according to claim 15 or 16.

45) A nucleic acid according to claims 1-16, a DNA fragment according to claim 17, a recombinant DNA molecule according to claim 18, a live recombinant carrier according to claim 19, a host cell according to claim 20 or a protein according to claims 21-44 or an immunogenic fragment thereof, for use in a vaccine.

46) Use of a nucleic acid according to claims 1-16, a DNA fragment according to claim 17, a recombinant DNA molecule according to claim 18, a live recombinant carrier according to claim 19, a host cell according to claim 20 or a protein according to claims 21-44 or an immunogenic fragment thereof for the manufacturing of a vaccine for combating *Ornithobacterium rhinotracheale* infection.

47) Vaccine for combating *Ornithobacterium rhinotracheale* infection, characterized in that it comprises a nucleic acid according to claims 1-16, a DNA fragment according to claim 17, a recombinant DNA molecule according to claim 18, a live recombinant carrier according to claim 19, a host cell according to claim 20 or a protein according to claims 21-44 or an immunogenic fragment thereof, and a pharmaceutically acceptable carrier.

48) Vaccine for combating *Ornithobacterium rhinotracheale* infection, characterized in that it comprises antibodies against a protein according to claims 21-44 or an immunogenic fragment of said protein, and a pharmaceutically acceptable carrier.

49) Vaccine according to claim 47, characterized in that it comprises an adjuvant.

50) Vaccine according to claim 47-49, characterized in that it comprises an additional antigen derived from a virus or micro-organism pathogenic to poultry, an antibody against such an antigen or genetic information encoding said antigen.

51) Vaccine according to claim 50, characterized in that said virus or micro-organism pathogenic to chickens is selected from the group consisting of Fowlpox virus, Infectious Bronchitis virus, Infectious Bursal Disease (Gumboro), Marek's Disease Virus, Chicken Anaemia agent, Avian Reovirus, *Mycoplasma gallisepticum*, Turkey Rhinotracheitis virus, *Haemophilus paragallinarum* (Coryza), Chicken Poxvirus, Avian Encephalomyelitisvirus, Duck Plague virus, Newcastle Disease virus, Egg Drop syndrome virus, Infectious Laryngotracheitis virus, Herpes Virus of Turkeys, Eimeria species, *Ornithobacterium rhinotracheale, Pasteurella multocida, Mycoplasma synoviae, Salmonella* species and *E. coli.*

52) Method for the preparation of a vaccine according to claims 47-51, said method comprising the admixing of a nucleic acid according to claims 1-16, a DNA fragment according to claim 17, a recombinant DNA molecule according to claim 18, a live recombinant carrier according to claim 19, a host cell according to claim 20, a protein according to claims 21-44 or an immunogenic fragment thereof, or antibodies against a protein according to claims 21-44 and a pharmaceutically acceptable carrier.

Abstract

The present invention relates to nucleic acids encoding *Ornithobacterium rhinotracheale* proteins, to DNA fragments, recombinant DNA molecules, live recombinant carriers and to host cells comprising such nucleic acids. The present invention also relates to *Ornithobacterium rhinotracheale* proteins and to antibodies against such proteins. Another embodiment of the invention relates to such proteins for use in vaccines and to the use of such proteins in the manufacturing of such vaccines. Also an embodiment of the invention relates to vaccines comprising such nucleic acids, DNA fragments, recombinant DNA molecules, live recombinant carriers, host cells, proteins or antibodies against such proteins. Finally, again another embodiment of the invention relates to methods for the preparation of such vaccines.

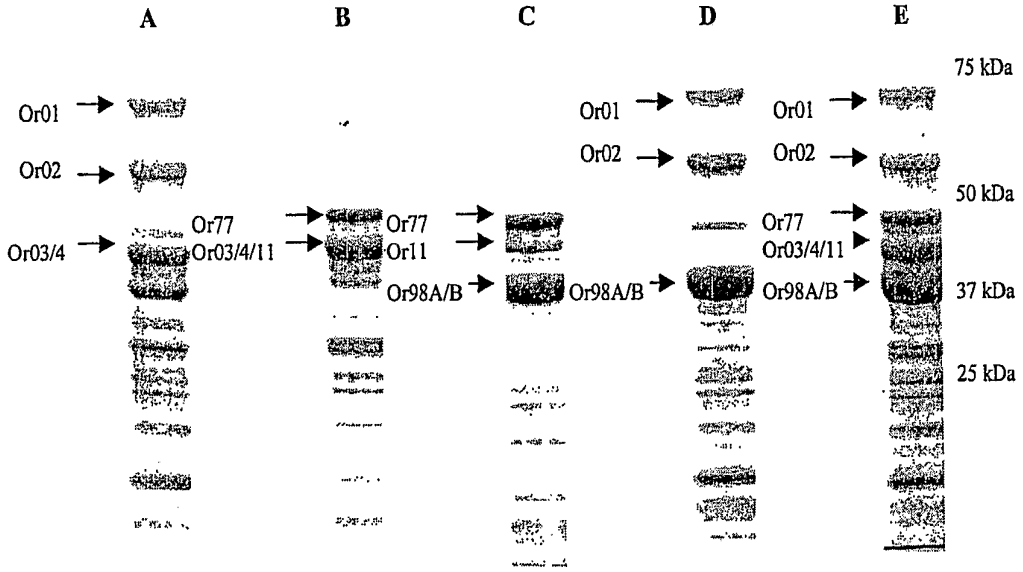


Figure 1.

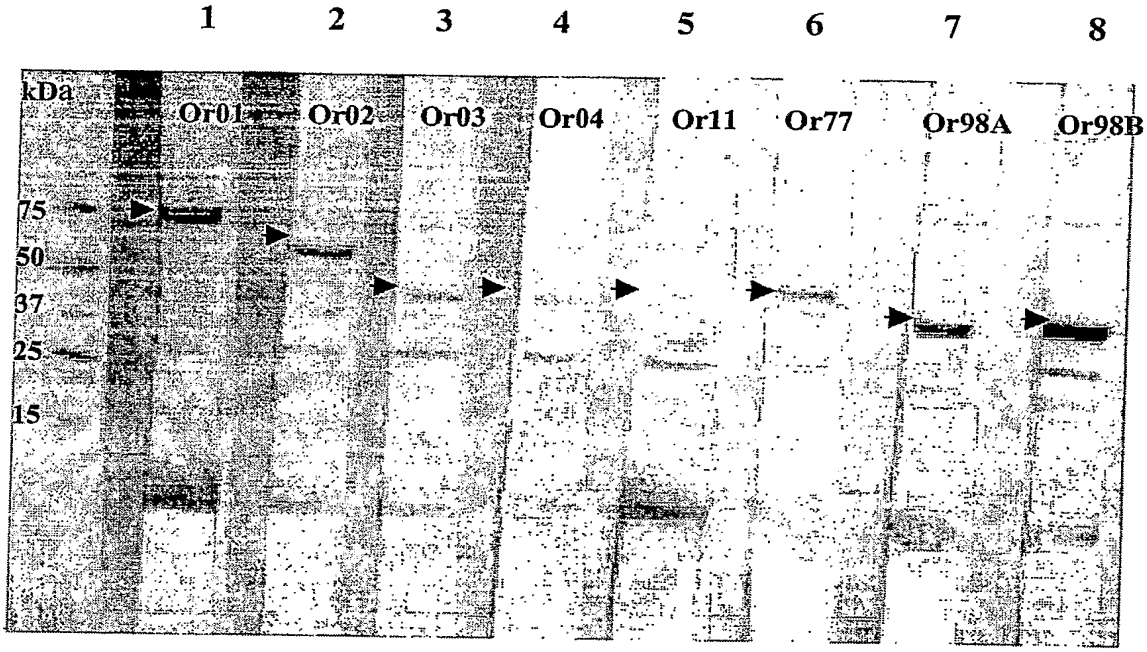
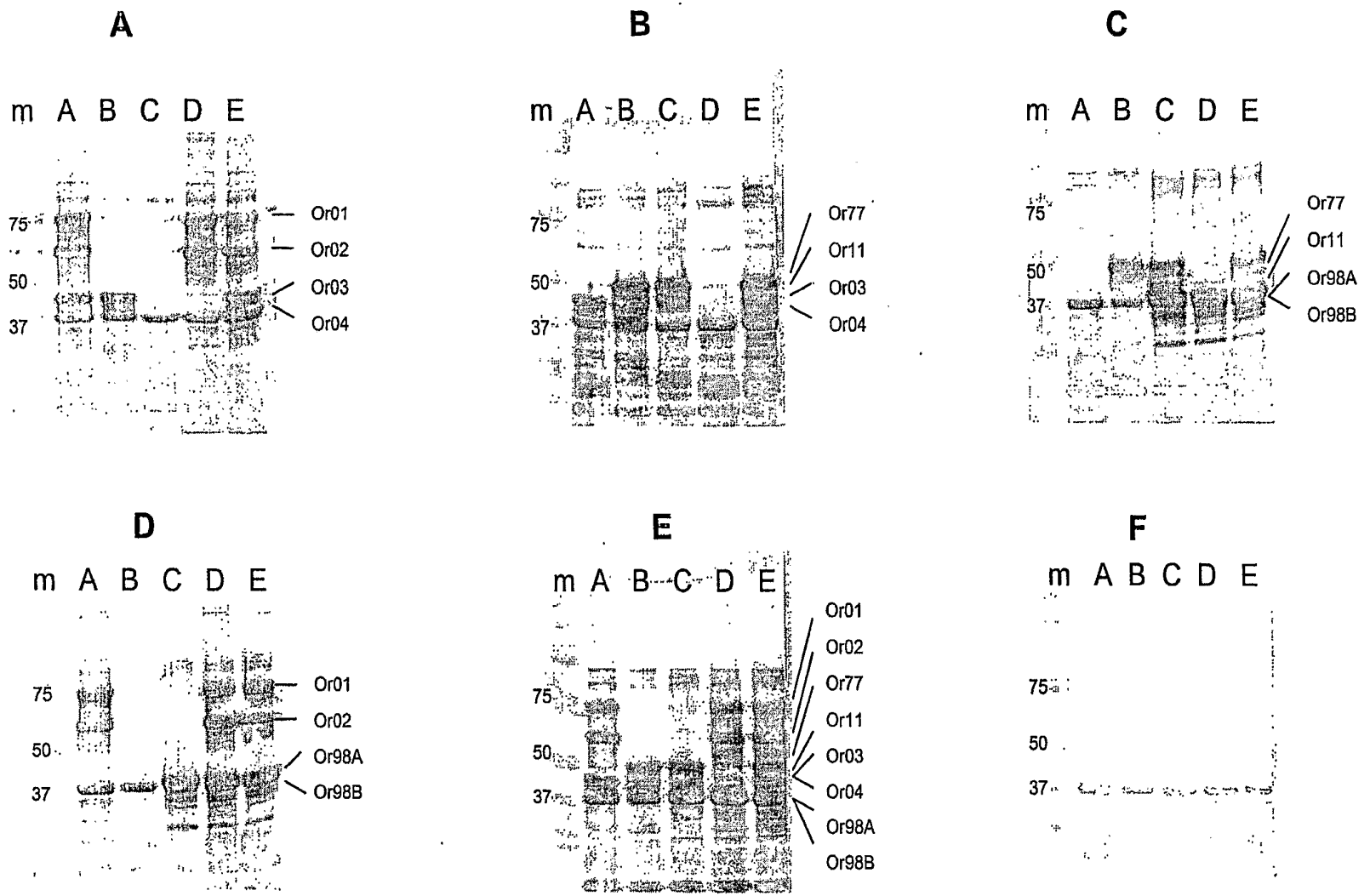


Figure 2.

Strip	Protein on blot:	Sera from birds vaccinated with:
1	Or01	Or01
2	Or02	Or02
3	Or03	Or03
4	Or04	Or04
5	Or11	Or11
6	Or77	Or77
7	Or98A	Or98A
8	Or98B	Or98B

Figure 3.


A
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75
50
37
Or01
Or02
Or03
Or04
B
m A B C D E
75
50
37
Or77
Or11
Or03
Or04
C
m A B C D E
75
50
37
Or77
Or11
Or98A
Or98B
D
m A B C D E
75
50
37
Or01
Or02
Or98A
Or98B
E
m A B C D E
75
50
37
Or01
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Or77
Or11
Or03
Or04
Or98A
Or98B
F
m A B C D E
75
50
37

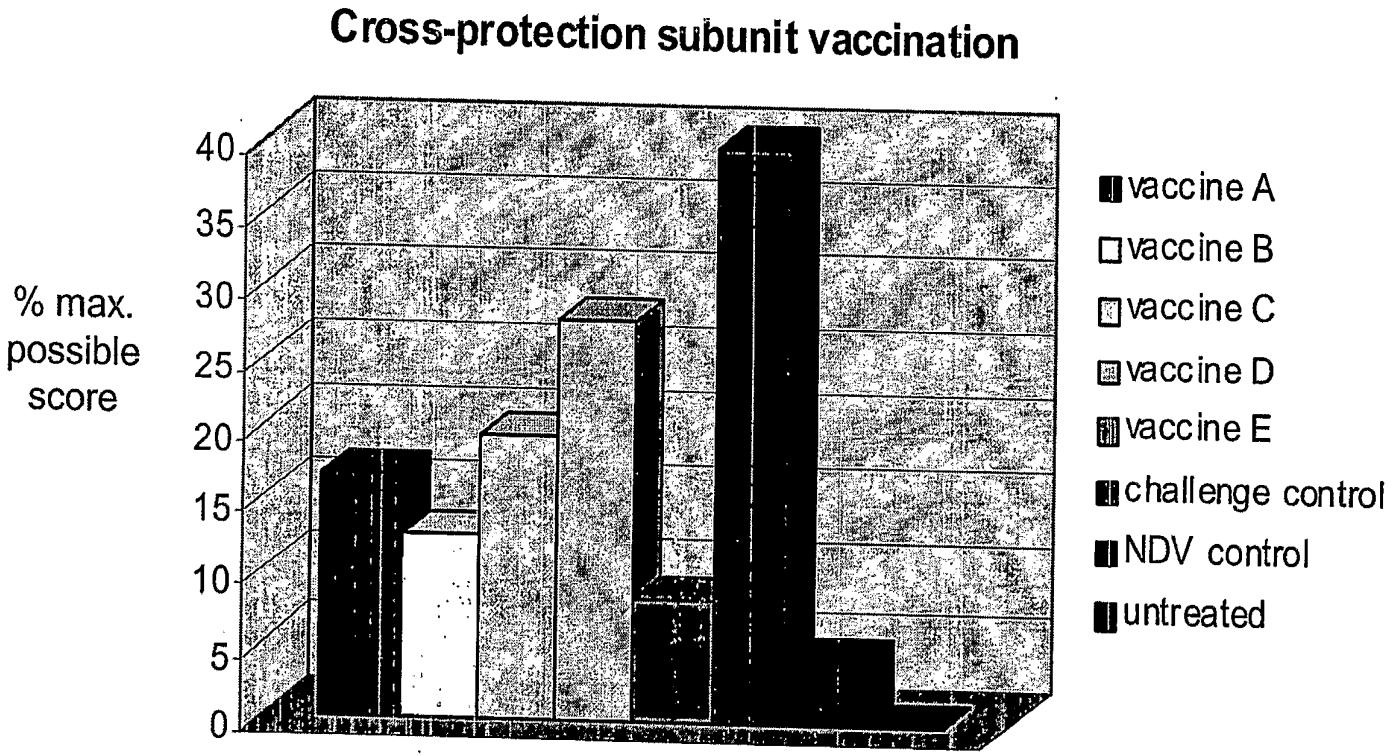
Cross-protection subunit vaccination
% max. possible score
40
35
30
25
20
15
10
5
0
vaccine A
vaccine B
vaccine C
vaccine D
vaccine E
challenge control
NDV control
untreated

Figure 4.

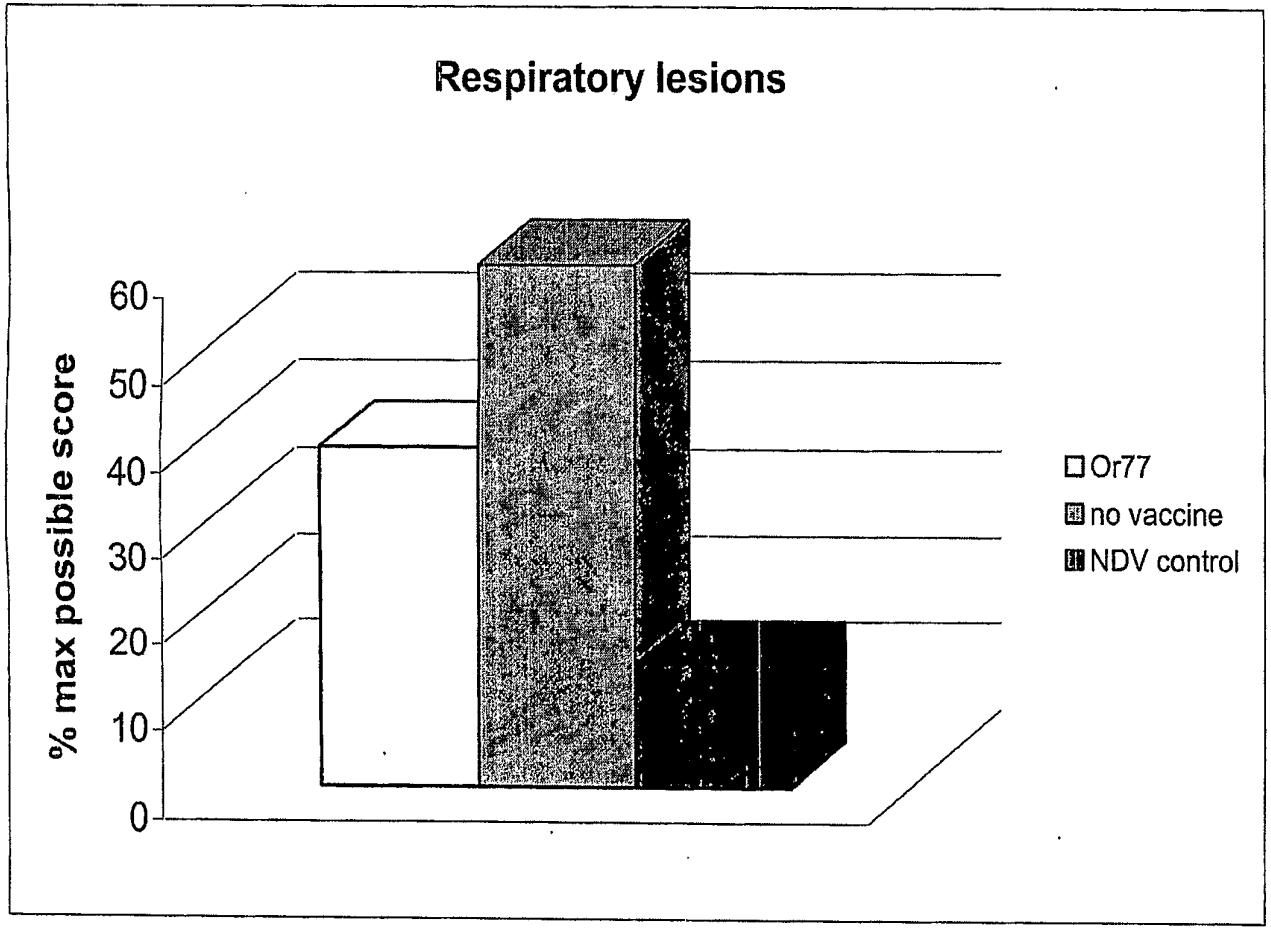
Respiratory lesions
% max possible score
60
50
40
30
20
10
0
Or77
no vaccine
NDV control

Figure 5.

SEQUENCE LISTING

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<130> 2004.011

<160> 16

<170> PatentIn version 3.2

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ggt aaa gtg gaa tct tgg aac aaa aaa gta gga gat aaa gta tca tac       96
Gly Lys Val Glu Ser Trp Asn Lys Lys Val Gly Asp Lys Val Ser Tyr
            20                  25                  30

ggc gac atc tta gcc gaa atc gaa aca gat aaa gcg gtt caa gaa ttt      144
Gly Asp Ile Leu Ala Glu Ile Glu Thr Asp Lys Ala Val Gln Glu Phe
        35                  40                  45

gaa aca gat gta gaa ggt act ctt tta tac atc ggt gta gag gct ggt      192
Glu Thr Asp Val Glu Gly Thr Leu Leu Tyr Ile Gly Val Glu Ala Gly
    50                  55                  60

caa gca gca cca gtt gat agt att tta gct atc atc ggt gca gaa ggc      240
Gln Ala Ala Pro Val Asp Ser Ile Leu Ala Ile Ile Gly Ala Glu Gly
65                  70                  75                  80

gaa gac atc agc ggt ttg gta agc ggt gga ggt gct agc caa tca gcg      288
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100 105 110

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Ala Pro Ala Ala Glu Val Pro Glu Asn Val Thr Ile Val Ser Met Pro
115 120 125

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Arg Leu Ser Asp Thr Met Glu Glu Gly Lys Val Glu Ser Trp Asn Lys
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Lys Val Gly Asp Lys Val Ser Tyr Gly Asp Ile Leu Ala Glu Ile Glu
145 150 155 160

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Thr Asp Lys Ala Val Gln Glu Phe Glu Thr Asp Val Glu Gly Thr Leu
165 170 175

tta tat ata ggt gta gaa gct ggg caa tca gca cca gtt gat agc att 576
Leu Tyr Ile Gly Val Glu Ala Gly Gln Ser Ala Pro Val Asp Ser Ile
180 185 190

ttg gca atc atc gga cct gaa gga aca gat gtt tct gca atc gta gca 624
Leu Ala Ile Ile Gly Pro Glu Gly Thr Asp Val Ser Ala Ile Val Ala
195 200 205

gga ggt ggt gca aaa cca gct gct aaa gcg gaa gct cca aag gct gaa 672
Gly Gly Gly Ala Lys Pro Ala Ala Lys Ala Glu Ala Pro Lys Ala Glu
210 215 220

gca cct aag caa gct gct cca gca caa gag aaa aaa gaa act cca gcg 720
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cct gct gct cca aaa gca caa gct acc aac aat tca ggt aga gta ttt 768
Pro Ala Ala Pro Lys Ala Gln Ala Thr Asn Asn Ser Gly Arg Val Phe
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Ile Ser Pro Leu Ala Lys Lys Leu Ala Asp Glu Lys Gly Tyr Asp Ile

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        275                 280                 285

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Val Glu Asn Phe Thr Pro Gln Ala Ala Ala Ala Lys Pro Ala Val Ala
    290                 295                 300

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305                 310                 315                 320

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    370                 375                 380

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385                 390                 395                 400

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            420                 425                 430

gta aaa gat tta gct aca aga tca aga gat aga aaa atc aaa gct gat     1344
Val Lys Asp Leu Ala Thr Arg Ser Arg Asp Arg Lys Ile Lys Ala Asp
        435                 440                 445
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Ala Pro Ala Ala Glu Val Pro Glu Asn Val Thr Ile Val Ser Met Pro
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Arg Leu Ser Asp Thr Met Glu Glu Gly Lys Val Glu Ser Trp Asn Lys
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405 410 415

Val Val Lys Asn Thr Asp Leu Lys Ser Leu Ser Gln Ile Ser Ala Glu
420 425 430

Val Lys Asp Leu Ala Thr Arg Ser Arg Asp Arg Lys Ile Lys Ala Asp
435 440 445

Glu Met Glu Gly Ser Thr Phe Thr Val Ser Asn Leu Gly Ala Tyr Gly
450 455 460

Val Glu Ser Phe Thr Ser Ile Ile Asn Gln Pro Asn Ser Cys Ile Leu
465 470 475 480

Ser Val Gly Ala Ile Val Glu Lys Pro Val Val Lys Asn Gly Gln Ile
485 490 495

Val Val Gly His Thr Met Lys Leu Cys Leu Ala Cys Asp His Arg Thr
500 505 510

Val Asp Gly Ala Thr Gly Ser Thr Phe Leu Gln Thr Leu Lys Gln Tyr
515 520 525

Leu Glu Thr Pro Met Ser Met Leu Val
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1               5                  10                  15

ttt gca gca tgt agc gat ttt gat tac aat gta gaa aac cca aac ctc      96
Phe Ala Ala Cys Ser Asp Phe Asp Tyr Asn Val Glu Asn Pro Asn Leu
            20                  25                  30

acg aag gga gag gct gat ttc tct aaa tat gta gct tta gga aat tct     144
Thr Lys Gly Glu Ala Asp Phe Ser Lys Tyr Val Ala Leu Gly Asn Ser
        35                  40                  45

ctc act tct ggt tat tca gac gga gcc tta tat cgc tcg gca caa gag     192
Leu Thr Ser Gly Tyr Ser Asp Gly Ala Leu Tyr Arg Ser Ala Gln Glu
    50                  55                  60

aat tca tac ccc gca atc att gcc aaa caa atg aaa tat gta ggc ggt     240
Asn Ser Tyr Pro Ala Ile Ile Ala Lys Gln Met Lys Tyr Val Gly Gly
65                  70                  75                  80

ggc gag ttc tct caa cct ttg atg aaa gac aac att ggt ggt ttt tcg     288
Gly Glu Phe Ser Gln Pro Leu Met Lys Asp Asn Ile Gly Gly Phe Ser
                85                  90                  95

gat ttg ttt gaa gca agt aaa cac acc gca ttt tac gga aaa tta gaa     336
Asp Leu Phe Glu Ala Ser Lys His Thr Ala Phe Tyr Gly Lys Leu Glu
            100                 105                 110

tta aaa atc gta gac ggt gca cct acg cca gtg cct tct gtg cct aag     384
Leu Lys Ile Val Asp Gly Ala Pro Thr Pro Val Pro Ser Val Pro Lys
        115                 120                 125

ttt agt tta gct caa acc ttc gta aaa ggg aat ttt aat aat ttg ggc     432
Phe Ser Leu Ala Gln Thr Phe Val Lys Gly Asn Phe Asn Asn Leu Gly
    130                 135                 140

gtg cca ggg gct aaa tct tat cat tta tta gct caa ggt tac gga aat     480
Val Pro Gly Ala Lys Ser Tyr His Leu Leu Ala Gln Gly Tyr Gly Asn
145                 150                 155                 160

att gct aat ctg aag gag agt aaa gcc aat cca tat ttt gtg cga ttt     528
Ile Ala Asn Leu Lys Glu Ser Lys Ala Asn Pro Tyr Phe Val Arg Phe
                165                 170                 175

gct agc caa cca aat gcc agc gtg ctg agc gat gct ttg gca caa aaa     576
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Ala Ser Gln Pro Asn Ala Ser Val Leu Ser Asp Ala Leu Ala Gln Lys
            180                 185                 190

cct aca ttc ttt acc tta tgg atc ggg aac aac gat gtt tta ggc tat      624
Pro Thr Phe Phe Thr Leu Trp Ile Gly Asn Asn Asp Val Leu Gly Tyr
        195                 200                 205

gcc atg aat ggc gca gca agc aca gat cga aaa ggg aac cct gat gta      672
Ala Met Asn Gly Ala Ala Ser Thr Asp Arg Lys Gly Asn Pro Asp Val
    210                 215                 220

aca aca tat aat tca aat gat ttg tct gat gct aac ttg gtg gca ggc      720
Thr Thr Tyr Asn Ser Asn Asp Leu Ser Asp Ala Asn Leu Val Ala Gly
225                 230                 235                 240

tct att caa aaa tta gta aaa gca ctt aca gat tca ggc gca aaa ggt      768
Ser Ile Gln Lys Leu Val Lys Ala Leu Thr Asp Ser Gly Ala Lys Gly
            245                 250                 255

gct gta gcg aat ttg cct tat gtc gaa gac att ccg tat ttt aca acc      816
Ala Val Ala Asn Leu Pro Tyr Val Glu Asp Ile Pro Tyr Phe Thr Thr
            260                 265                 270

gtg ccg gct gag cct tta agc cct tta aac aaa agt tac gct aca caa      864
Val Pro Ala Glu Pro Leu Ser Pro Leu Asn Lys Ser Tyr Ala Thr Gln
        275                 280                 285

att gaa aat ttg aat aaa ttt tat gct agc cta aat aaa gtt ttt gat      912
Ile Glu Asn Leu Asn Lys Phe Tyr Ala Ser Leu Asn Lys Val Phe Asp
    290                 295                 300

gcc cta gga gca agc gat aga aaa atc aca ttt aat gcc gat aaa gca      960
Ala Leu Gly Ala Ser Asp Arg Lys Ile Thr Phe Asn Ala Asp Lys Ala
305                 310                 315                 320

agc ggt gct gtg att gta gat aaa agt ttg cca gat tta agt caa aaa     1008
Ser Gly Ala Val Ile Val Asp Lys Ser Leu Pro Asp Leu Ser Gln Lys
                325                 330                 335

atc tta gca acc tta cta aaa tta gaa ttc cca aac gaa aaa gct aaa     1056
Ile Leu Ala Thr Leu Leu Lys Leu Glu Phe Pro Asn Glu Lys Ala Lys
            340                 345                 350

ctt tta gca caa acc ttt ggt caa gtg cgc caa tct aaa gca gga gat     1104
Leu Leu Ala Gln Thr Phe Gly Gln Val Arg Gln Ser Lys Ala Gly Asp
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355 360 365

tta ttg cca ctt aca gcg agt aga aca ctt ggg aaa tta aat agt gaa 1152
Leu Leu Pro Leu Thr Ala Ser Arg Thr Leu Gly Lys Leu Asn Ser Glu
370 375 380

aga ctt gct act ttg aca aaa tta gga tta cca aag gaa aac gcc gct 1200
Arg Leu Ala Thr Leu Thr Lys Leu Gly Leu Pro Lys Glu Asn Ala Ala
385 390 395 400

caa ctt tct atg aac gga ctt act tat cca ttg caa gat gcc gat gtt 1248
Gln Leu Ser Met Asn Gly Leu Thr Tyr Pro Leu Gln Asp Ala Asp Val
405 410 415

tta acc aaa aat gaa gtt tca aca att cac gaa aga gta aac gaa atc 1296
Leu Thr Lys Asn Glu Val Ser Thr Ile His Glu Arg Val Asn Glu Ile
420 425 430

aat caa ggc ata caa gca gtg gca aaa caa ttc aac att gca tat gtg 1344
Asn Gln Gly Ile Gln Ala Val Ala Lys Gln Phe Asn Ile Ala Tyr Val
435 440 445

gac atg aat gcc gaa atg caa aaa ctc act aaa ggc ttt aaa ttc aac 1392
Asp Met Asn Ala Glu Met Gln Lys Leu Thr Lys Gly Phe Lys Phe Asn
450 455 460

ggg gta gac tac aac gca agt ttt gtg act ggt gga gct ttt tcg ctt 1440
Gly Val Asp Tyr Asn Ala Ser Phe Val Thr Gly Gly Ala Phe Ser Leu
465 470 475 480

gat gga gtg cat tta aac agc cga gga tat gcc cat aca gct aat aca 1488
Asp Gly Val His Leu Asn Ser Arg Gly Tyr Ala His Thr Ala Asn Thr
485 490 495

ttt att cgt gcc atc aat cag caa tat aag gca agc att ccg ttg gta 1536
Phe Ile Arg Ala Ile Asn Gln Gln Tyr Lys Ala Ser Ile Pro Leu Val
500 505 510

gat atc aac gct ttc cca ggc aca caa tta cct taa 1572
Asp Ile Asn Ala Phe Pro Gly Thr Gln Leu Pro
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<213> *Ornithobacterium rhinotracheale*

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Phe Ala Ala Cys Ser Asp Phe Asp Tyr Asn Val Glu Asn Pro Asn Leu
            20                  25                  30

Thr Lys Gly Glu Ala Asp Phe Ser Lys Tyr Val Ala Leu Gly Asn Ser
        35                  40                  45

Leu Thr Ser Gly Tyr Ser Asp Gly Ala Leu Tyr Arg Ser Ala Gln Glu
    50                  55                  60

Asn Ser Tyr Pro Ala Ile Ile Ala Lys Gln Met Lys Tyr Val Gly Gly
65                  70                  75                  80

Gly Glu Phe Ser Gln Pro Leu Met Lys Asp Asn Ile Gly Gly Phe Ser
                85                  90                  95

Asp Leu Phe Glu Ala Ser Lys His Thr Ala Phe Tyr Gly Lys Leu Glu
            100                 105                 110

Leu Lys Ile Val Asp Gly Ala Pro Thr Pro Val Pro Ser Val Pro Lys
        115                 120                 125

Phe Ser Leu Ala Gln Thr Phe Val Lys Gly Asn Phe Asn Asn Leu Gly
    130                 135                 140

Val Pro Gly Ala Lys Ser Tyr His Leu Leu Ala Gln Gly Tyr Gly Asn
145                 150                 155                 160
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Ile Ala Asn Leu Lys Glu Ser Lys Ala Asn Pro Tyr Phe Val Arg Phe
165 170 175

Ala Ser Gln Pro Asn Ala Ser Val Leu Ser Asp Ala Leu Ala Gln Lys
180 185 190

Pro Thr Phe Phe Thr Leu Trp Ile Gly Asn Asn Asp Val Leu Gly Tyr
195 200 205

Ala Met Asn Gly Ala Ala Ser Thr Asp Arg Lys Gly Asn Pro Asp Val
210 215 220

Thr Thr Tyr Asn Ser Asn Asp Leu Ser Asp Ala Asn Leu Val Ala Gly
225 230 235 240

Ser Ile Gln Lys Leu Val Lys Ala Leu Thr Asp Ser Gly Ala Lys Gly
245 250 255

Ala Val Ala Asn Leu Pro Tyr Val Glu Asp Ile Pro Tyr Phe Thr Thr
260 265 270

Val Pro Ala Glu Pro Leu Ser Pro Leu Asn Lys Ser Tyr Ala Thr Gln
275 280 285

Ile Glu Asn Leu Asn Lys Phe Tyr Ala Ser Leu Asn Lys Val Phe Asp
290 295 300

Ala Leu Gly Ala Ser Asp Arg Lys Ile Thr Phe Asn Ala Asp Lys Ala
305 310 315 320

Ser Gly Ala Val Ile Val Asp Lys Ser Leu Pro Asp Leu Ser Gln Lys
325 330 335

Ile Leu Ala Thr Leu Leu Lys Leu Glu Phe Pro Asn Glu Lys Ala Lys

340 345 350

Leu Leu Ala Gln Thr Phe Gly Gln Val Arg Gln Ser Lys Ala Gly Asp
355 360 365

Leu Leu Pro Leu Thr Ala Ser Arg Thr Leu Gly Lys Leu Asn Ser Glu
370 375 380

Arg Leu Ala Thr Leu Thr Lys Leu Gly Leu Pro Lys Glu Asn Ala Ala
385 390 395 400

Gln Leu Ser Met Asn Gly Leu Thr Tyr Pro Leu Gln Asp Ala Asp Val
405 410 415

Leu Thr Lys Asn Glu Val Ser Thr Ile His Glu Arg Val Asn Glu Ile
420 425 430

Asn Gln Gly Ile Gln Ala Val Ala Lys Gln Phe Asn Ile Ala Tyr Val
435 440 445

Asp Met Asn Ala Glu Met Gln Lys Leu Thr Lys Gly Phe Lys Phe Asn
450 455 460

Gly Val Asp Tyr Asn Ala Ser Phe Val Thr Gly Gly Ala Phe Ser Leu
465 470 475 480

Asp Gly Val His Leu Asn Ser Arg Gly Tyr Ala His Thr Ala Asn Thr
485 490 495

Phe Ile Arg Ala Ile Asn Gln Gln Tyr Lys Ala Ser Ile Pro Leu Val
500 505 510

Asp Ile Asn Ala Phe Pro Gly Thr Gln Leu Pro
515 520

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Met Lys Lys Leu Ser Tyr Leu Leu Phe Ser Ile Pro Leu Leu Trp Gly
1               5                  10                  15

ttg aat gct tgt acc gaa gat ttt gaa ccc aca ttt tca gaa aac gca      96
Leu Asn Ala Cys Thr Glu Asp Phe Glu Pro Thr Phe Ser Glu Asn Ala
            20                  25                  30

act cag cgt tat ata aat gtt cag aac gaa att aca gaa ttt ctt agt     144
Thr Gln Arg Tyr Ile Asn Val Gln Asn Glu Ile Thr Glu Phe Leu Ser
        35                  40                  45

acc cct gat gct gat ttt att tta caa tac ttc cca gat gat aat cag     192
Thr Pro Asp Ala Asp Phe Ile Leu Gln Tyr Phe Pro Asp Asp Asn Gln
    50                  55                  60

tct tat gga gga tat aat tat ttc ttg aaa ttc tca gga aaa gat aag     240
Ser Tyr Gly Gly Tyr Asn Tyr Phe Leu Lys Phe Ser Gly Lys Asp Lys
65                  70                  75                  80

gta agt gcg gaa tca gag acc aat gaa caa gct gta agt tct act ttt     288
Val Ser Ala Glu Ser Glu Thr Asn Glu Gln Ala Val Ser Ser Thr Phe
                85                  90                  95

cga att ctt caa aat gga ggt gca gtt ctt acc ttt gat tta tac aat     336
Arg Ile Leu Gln Asn Gly Gly Ala Val Leu Thr Phe Asp Leu Tyr Asn
            100                 105                 110

gag gag cta cat gaa ttt gca act cct agt cca tca gaa tat cgt gca     384
Glu Glu Leu His Glu Phe Ala Thr Pro Ser Pro Ser Glu Tyr Arg Ala
        115                 120                 125
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aaa cga gga gat ttt gaa ttt ttg atc ctt aaa aaa agt aat gac aca      432
Lys Arg Gly Asp Phe Glu Phe Leu Ile Leu Lys Lys Ser Asn Asp Thr
    130                 135                 140

ctt tat cta aaa gga aag aaa aca gga aat tac atg aag cta tat aaa      480
Leu Tyr Leu Lys Gly Lys Lys Thr Gly Asn Tyr Met Lys Leu Tyr Lys
145                 150                 155                 160

gca ggg aat att caa gag att aaa agt aac att aga aaa gta gca act      528
Ala Gly Asn Ile Gln Glu Ile Lys Ser Asn Ile Arg Lys Val Ala Thr
                165                 170                 175

aca att gat agg gta gat ctt cca gct caa ggt act ata ggt aca gag      576
Thr Ile Asp Arg Val Asp Leu Pro Ala Gln Gly Thr Ile Gly Thr Glu
            180                 185                 190

cct ttg gta ttg tca aca gga gga act aga aat att att ttt agt act      624
Pro Leu Val Leu Ser Thr Gly Gly Thr Arg Asn Ile Ile Phe Ser Thr
        195                 200                 205

tta aat ggg ggg agt ata gag tct aca gaa gca tcg tat att ttt aca      672
Leu Asn Gly Gly Ser Ile Glu Ser Thr Glu Ala Ser Tyr Ile Phe Thr
    210                 215                 220

gaa aac gga att aag ttt tac aaa cca gtt gaa att aag ggg aaa gtt      720
Glu Asn Gly Ile Lys Phe Tyr Lys Pro Val Glu Ile Lys Gly Lys Val
225                 230                 235                 240

tac ggt gga tta att ttt gac gaa agt act caa aca tta aag tca gaa      768
Tyr Gly Gly Leu Ile Phe Asp Glu Ser Thr Gln Thr Leu Lys Ser Glu
                245                 250                 255

gat ggt gta att gta att aat ttg aaa ttt gtt cct atc aac ttt aaa      816
Asp Gly Val Ile Val Ile Asn Leu Lys Phe Val Pro Ile Asn Phe Lys
            260                 265                 270

tca aaa gct tgg ttt ttg gat atg agc aaa tca gag aat aca tcg gaa      864
Ser Lys Ala Trp Phe Leu Asp Met Ser Lys Ser Glu Asn Thr Ser Glu
        275                 280                 285

ggt tat aag aaa gcc aga gca ggc gat agt ctt ttg cat ggt atg att      912
Gly Tyr Lys Lys Ala Arg Ala Gly Asp Ser Leu Leu His Gly Met Ile
    290                 295                 300

cta agt aaa ttt aag tta caa gat ttc tat gtg tta ggt aat ttt aga      960
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Leu Ser Lys Phe Lys Leu Gln Asp Phe Tyr Val Leu Gly Asn Phe Arg
305 310 315 320

gat aat gta gga ttc aat act ttt gtt gag ggc tat aac gga gca ttt 1008
Asp Asn Val Gly Phe Asn Thr Phe Val Glu Gly Tyr Asn Gly Ala Phe
325 330 335

gca att tat ggt tta agt ttc aaa gga gaa gat tca aat cca aat ctt 1056
Ala Ile Tyr Gly Leu Ser Phe Lys Gly Glu Asp Ser Asn Pro Asn Leu
340 345 350

atc cac att gag aaa aca aaa cct gtt gaa ttt gat gct tat ttc aaa 1104
Ile His Ile Glu Lys Thr Lys Pro Val Glu Phe Asp Ala Tyr Phe Lys
355 360 365

tat gtg aat gga gtt tta gat aaa atc act aaa aat tca cct tat att 1152
Tyr Val Asn Gly Val Leu Asp Lys Ile Thr Lys Asn Ser Pro Tyr Ile
370 375 380

gta gag gag gtt cag tca gat cct aaa cgt gtg aag cta ata agt aaa 1200
Val Glu Glu Val Gln Ser Asp Pro Lys Arg Val Lys Leu Ile Ser Lys
385 390 395 400

aat gat caa gaa tta tgg ttt att ctt gat ttg ctt aaa tga 1242
Asn Asp Gln Glu Leu Trp Phe Ile Leu Asp Leu Leu Lys
405 410

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<213> *Ornithobacterium rhinotracheale*

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Leu Asn Ala Cys Thr Glu Asp Phe Glu Pro Thr Phe Ser Glu Asn Ala
20 25 30

Thr Gln Arg Tyr Ile Asn Val Gln Asn Glu Ile Thr Glu Phe Leu Ser
35 40 45

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Thr Pro Asp Ala Asp Phe Ile Leu Gln Tyr Phe Pro Asp Asp Asn Gln
    50                  55                  60

Ser Tyr Gly Gly Tyr Asn Tyr Phe Leu Lys Phe Ser Gly Lys Asp Lys
65                  70                  75                  80

Val Ser Ala Glu Ser Glu Thr Asn Glu Gln Ala Val Ser Ser Thr Phe
                85                  90                  95

Arg Ile Leu Gln Asn Gly Gly Ala Val Leu Thr Phe Asp Leu Tyr Asn
            100                 105                 110

Glu Glu Leu His Glu Phe Ala Thr Pro Ser Pro Ser Glu Tyr Arg Ala
        115                 120                 125

Lys Arg Gly Asp Phe Glu Phe Leu Ile Leu Lys Lys Ser Asn Asp Thr
    130                 135                 140

Leu Tyr Leu Lys Gly Lys Lys Thr Gly Asn Tyr Met Lys Leu Tyr Lys
145                 150                 155                 160

Ala Gly Asn Ile Gln Glu Ile Lys Ser Asn Ile Arg Lys Val Ala Thr
                165                 170                 175

Thr Ile Asp Arg Val Asp Leu Pro Ala Gln Gly Thr Ile Gly Thr Glu
            180                 185                 190

Pro Leu Val Leu Ser Thr Gly Gly Thr Arg Asn Ile Ile Phe Ser Thr
        195                 200                 205

Leu Asn Gly Gly Ser Ile Glu Ser Thr Glu Ala Ser Tyr Ile Phe Thr
    210                 215                 220
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Glu Asn Gly Ile Lys Phe Tyr Lys Pro Val Glu Ile Lys Gly Lys Val
225 230 235 240

Tyr Gly Gly Leu Ile Phe Asp Glu Ser Thr Gln Thr Leu Lys Ser Glu
245 250 255

Asp Gly Val Ile Val Ile Asn Leu Lys Phe Val Pro Ile Asn Phe Lys
260 265 270

Ser Lys Ala Trp Phe Leu Asp Met Ser Lys Ser Glu Asn Thr Ser Glu
275 280 285

Gly Tyr Lys Lys Ala Arg Ala Gly Asp Ser Leu Leu His Gly Met Ile
290 295 300

Leu Ser Lys Phe Lys Leu Gln Asp Phe Tyr Val Leu Gly Asn Phe Arg
305 310 315 320

Asp Asn Val Gly Phe Asn Thr Phe Val Glu Gly Tyr Asn Gly Ala Phe
325 330 335

Ala Ile Tyr Gly Leu Ser Phe Lys Gly Glu Asp Ser Asn Pro Asn Leu
340 345 350

Ile His Ile Glu Lys Thr Lys Pro Val Glu Phe Asp Ala Tyr Phe Lys
355 360 365

Tyr Val Asn Gly Val Leu Asp Lys Ile Thr Lys Asn Ser Pro Tyr Ile
370 375 380

Val Glu Glu Val Gln Ser Asp Pro Lys Arg Val Lys Leu Ile Ser Lys
385 390 395 400

Asn Asp Gln Glu Leu Trp Phe Ile Leu Asp Leu Leu Lys
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ctc acc aat tgc tat gat agc gat gag att gaa gta att aaa ttt gat 96
Leu Thr Asn Cys Tyr Asp Ser Asp Glu Ile Glu Val Ile Lys Phe Asp
20 25 30

gat tct ttt act cca gct ccg ccc acc gaa aaa aaa aga gac act ccg 144
Asp Ser Phe Thr Pro Ala Pro Pro Thr Glu Lys Lys Arg Asp Thr Pro
35 40 45

cta ata aat tta tta gat gat ttt gta ttc ttt aaa aaa gat gta gta 192
Leu Ile Asn Leu Leu Asp Asp Phe Val Phe Phe Lys Lys Asp Val Val
50 55 60

aca att ccg gta gat aaa gac aat tta gcc acc aat aat gtc atc agt 240
Thr Ile Pro Val Asp Lys Asp Asn Leu Ala Thr Asn Asn Val Ile Ser
65 70 75 80

ggt gaa gtc ttt aca aat aga aaa atg tct gaa aat ttt gag tat cag 288
Gly Glu Val Phe Thr Asn Arg Lys Met Ser Glu Asn Phe Glu Tyr Gln
85 90 95

ctt gaa tta gac caa gat tgg att agt agc aat ccg gac tta caa gcc 336
Leu Glu Leu Asp Gln Asp Trp Ile Ser Ser Asn Pro Asp Leu Gln Ala
100 105 110

att cca aac gga gct ttt aca atc tct gga caa aca ctc aac aaa gat 384
Ile Pro Asn Gly Ala Phe Thr Ile Ser Gly Gln Thr Leu Asn Lys Asp

115 120 125

gaa aga aat ggt act ttc aaa att cag ctt aat gca gag gtg gcg aaa 432
Glu Arg Asn Gly Thr Phe Lys Ile Gln Leu Asn Ala Glu Val Ala Lys
130 135 140

gag cta gga ggc acc tac tat ctc ccg cta aaa ttg gtt tct aaa aat 480
Glu Leu Gly Gly Thr Tyr Tyr Leu Pro Leu Lys Leu Val Ser Lys Asn
145 150 155 160

gat aat tta aac att tta aag gga tat gaa agt ggc gtt ttt aag cta 528
Asp Asn Leu Asn Ile Leu Lys Gly Tyr Glu Ser Gly Val Phe Lys Leu
165 170 175

gta ttc aaa aaa tcg tat cca atc cca gaa ggt aac aat gtt gaa gga 576
Val Phe Lys Lys Ser Tyr Pro Ile Pro Glu Gly Asn Asn Val Glu Gly
180 185 190

aaa aaa gga tat tat ttt gat ggt tta ggc aat aat ata cct aga aca 624
Lys Lys Gly Tyr Tyr Phe Asp Gly Leu Gly Asn Asn Ile Pro Arg Thr
195 200 205

gat tta tcg ttt aat tca aat tac gcc ccc gat cat ctt ttt aaa tta 672
Asp Leu Ser Phe Asn Ser Asn Tyr Ala Pro Asp His Leu Phe Lys Leu
210 215 220

aat gat gga aac caa caa ggg gct aat tgg tgg gca gac act gat gat 720
Asn Asp Gly Asn Gln Gln Gly Ala Asn Trp Trp Ala Asp Thr Asp Asp
225 230 235 240

aac aca aca tat ctt gat gta aaa ttc cct att aat aca ata aaa gct 768
Asn Thr Thr Tyr Leu Asp Val Lys Phe Pro Ile Asn Thr Ile Lys Ala
245 250 255

ata aaa tta tac act aaa agc tat tgg caa aat gct gta ggc agt gta 816
Ile Lys Leu Tyr Thr Lys Ser Tyr Trp Gln Asn Ala Val Gly Ser Val
260 265 270

aaa att gaa gtt tct aat gat aat ggc aat act tgg aaa gaa cag gga 864
Lys Ile Glu Val Ser Asn Asp Asn Gly Asn Thr Trp Lys Glu Gln Gly
275 280 285

att gct aac ttt ggg caa tat tca aca gtg tct act att gta ttc act 912
Ile Ala Asn Phe Gly Gln Tyr Ser Thr Val Ser Thr Ile Val Phe Thr
290 295 300

caa cca att gac att aat gct gtc aga ata tct aac ttc act aga ggg 960
Gln Pro Ile Asp Ile Asn Ala Val Arg Ile Ser Asn Phe Thr Arg Gly
305 310 315 320

gga agt agt aat ttc att aac att aac gag gtg gaa gta ttc aaa ata 1008
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325 330 335

cca agt gaa gaa taa 1023
Pro Ser Glu Glu
340

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<211> 340
<212> PRT
<213> *Ornithobacterium rhinotracheale*

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Leu Thr Asn Cys Tyr Asp Ser Asp Glu Ile Glu Val Ile Lys Phe Asp
20 25 30

Asp Ser Phe Thr Pro Ala Pro Pro Thr Glu Lys Lys Arg Asp Thr Pro
35 40 45

Leu Ile Asn Leu Leu Asp Asp Phe Val Phe Phe Lys Lys Asp Val Val
50 55 60

Thr Ile Pro Val Asp Lys Asp Asn Leu Ala Thr Asn Asn Val Ile Ser
65 70 75 80

Gly Glu Val Phe Thr Asn Arg Lys Met Ser Glu Asn Phe Glu Tyr Gln
85 90 95

Leu Glu Leu Asp Gln Asp Trp Ile Ser Ser Asn Pro Asp Leu Gln Ala
100 105 110

Ile Pro Asn Gly Ala Phe Thr Ile Ser Gly Gln Thr Leu Asn Lys Asp
115 120 125

Glu Arg Asn Gly Thr Phe Lys Ile Gln Leu Asn Ala Glu Val Ala Lys
130 135 140

Glu Leu Gly Gly Thr Tyr Tyr Leu Pro Leu Lys Leu Val Ser Lys Asn
145 150 155 160

Asp Asn Leu Asn Ile Leu Lys Gly Tyr Glu Ser Gly Val Phe Lys Leu
165 170 175

Val Phe Lys Lys Ser Tyr Pro Ile Pro Glu Gly Asn Asn Val Glu Gly
180 185 190

Lys Lys Gly Tyr Tyr Phe Asp Gly Leu Gly Asn Asn Ile Pro Arg Thr
195 200 205

Asp Leu Ser Phe Asn Ser Asn Tyr Ala Pro Asp His Leu Phe Lys Leu
210 215 220

Asn Asp Gly Asn Gln Gln Gly Ala Asn Trp Trp Ala Asp Thr Asp Asp
225 230 235 240

Asn Thr Thr Tyr Leu Asp Val Lys Phe Pro Ile Asn Thr Ile Lys Ala
245 250 255

Ile Lys Leu Tyr Thr Lys Ser Tyr Trp Gln Asn Ala Val Gly Ser Val
260 265 270

Lys Ile Glu Val Ser Asn Asp Asn Gly Asn Thr Trp Lys Glu Gln Gly

275 280 285

Ile Ala Asn Phe Gly Gln Tyr Ser Thr Val Ser Thr Ile Val Phe Thr
290 295 300

Gln Pro Ile Asp Ile Asn Ala Val Arg Ile Ser Asn Phe Thr Arg Gly
305 310 315 320

Gly Ser Ser Asn Phe Ile Asn Ile Asn Glu Val Glu Val Phe Lys Ile
325 330 335

Pro Ser Glu Glu
340

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<212> DNA
<213> *Ornithobacterium rhinotracheale*

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Met Ile Lys Lys Val Phe Leu Ser Phe Val Leu Met Ala Ser Thr Gly
1 5 10 15

att tta tgg gca ggc gga tac cga gtt tcg ctg caa ggt gta aga caa 96
Ile Leu Trp Ala Gly Gly Tyr Arg Val Ser Leu Gln Gly Val Arg Gln
20 25 30

gcc gcc atg ggg gca caa ggt gta gca ctt tct cac gat gcg agt gtg 144
Ala Ala Met Gly Ala Gln Gly Val Ala Leu Ser His Asp Ala Ser Val
35 40 45

gca ttt ttc aac ccc gca gca ttg gct ttt gta gat gat aaa tta agt 192
Ala Phe Phe Asn Pro Ala Ala Leu Ala Phe Val Asp Asp Lys Leu Ser
50 55 60

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att gct gtg gga ggt ttc gga att ggg att acc gca aaa tac caa aac      240
Ile Ala Val Gly Gly Phe Gly Ile Gly Ile Thr Ala Lys Tyr Gln Asn
65                  70                  75                  80

cgc gaa acg ctc tat aaa gcc gaa acc gac aat ccg ctg ggg aca cca      288
Arg Glu Thr Leu Tyr Lys Ala Glu Thr Asp Asn Pro Leu Gly Thr Pro
                85                  90                  95

ctt tat ctt gct aca agc tat aag cct acg gaa aaa cta gcc tta ggc      336
Leu Tyr Leu Ala Thr Ser Tyr Lys Pro Thr Glu Lys Leu Ala Leu Gly
            100                 105                 110

gtg agc gta acc act ccg ttt ggg agc acc gta gac tgg gga gat aaa      384
Val Ser Val Thr Thr Pro Phe Gly Ser Thr Val Asp Trp Gly Asp Lys
        115                 120                 125

tgg gct gga cgc tac atc att gat aga att gcc ctc aaa tcg ttt ttt      432
Trp Ala Gly Arg Tyr Ile Ile Asp Arg Ile Ala Leu Lys Ser Phe Phe
    130                 135                 140

att cag ccc acg gca gcg tat aaa gta acc gat tgg ctc tct gtg ggg      480
Ile Gln Pro Thr Ala Ala Tyr Lys Val Thr Asp Trp Leu Ser Val Gly
145                 150                 155                 160

gct ggt gcc atc atc gct cga ggc aat gta aac att aag cgt gca ata      528
Ala Gly Ala Ile Ile Ala Arg Gly Asn Val Asn Ile Lys Arg Ala Ile
                165                 170                 175

tct cta ggc aac caa gat gcg ggg cta gaa atc gac aaa aaa gga gct      576
Ser Leu Gly Asn Gln Asp Ala Gly Leu Glu Ile Asp Lys Lys Gly Ala
            180                 185                 190

cac gga aca ggg ttt aat gta ggg gtt tat gcc aaa cca aat gat aaa      624
His Gly Thr Gly Phe Asn Val Gly Val Tyr Ala Lys Pro Asn Asp Lys
        195                 200                 205

tta aat ata gga att gct tac cga tca gaa gtg aag atg aaa gcg gac      672
Leu Asn Ile Gly Ile Ala Tyr Arg Ser Glu Val Lys Met Lys Ala Asp
    210                 215                 220

aaa ggt gat gct gtt ttc aaa aat tta cca agt atc gta aag ggc aaa      720
Lys Gly Asp Ala Val Phe Lys Asn Leu Pro Ser Ile Val Lys Gly Lys
225                 230                 235                 240
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atg cct ttt tcg gct aaa tat ttt gat gct caa tta cct cta cca gca      768
Met Pro Phe Ser Ala Lys Tyr Phe Asp Ala Gln Leu Pro Leu Pro Ala
                245                 250                 255

gaa ctt tta att ggg gcg aac tat aaa gta aca cca aaa ttg ctc gta      816
Glu Leu Leu Ile Gly Ala Asn Tyr Lys Val Thr Pro Lys Leu Leu Val
            260                 265                 270

ggg gca gaa att ggg gct gta aaa tgg aac gcc tac gaa aca tta aat      864
Gly Ala Glu Ile Gly Ala Val Lys Trp Asn Ala Tyr Glu Thr Leu Asn
        275                 280                 285

att aaa ctt tat aac aac gaa gag gaa tac aac aat act tct aac aaa      912
Ile Lys Leu Tyr Asn Asn Glu Glu Glu Tyr Asn Asn Thr Ser Asn Lys
    290                 295                 300

aat tac aaa aac aca tta aat tat agt atc ggg gct gaa tat tta atc      960
Asn Tyr Lys Asn Thr Leu Asn Tyr Ser Ile Gly Ala Glu Tyr Leu Ile
305                 310                 315                 320

aat cca aaa gct gcc tta cgc tta ggg tat aaa ttc gac aaa tcg cct     1008
Asn Pro Lys Ala Ala Leu Arg Leu Gly Tyr Lys Phe Asp Lys Ser Pro
                325                 330                 335

tcg cca gct gat tcg ttt aac cca gag acc cca acc att aat tat cac     1056
Ser Pro Ala Asp Ser Phe Asn Pro Glu Thr Pro Thr Ile Asn Tyr His
            340                 345                 350

gca ttt aca act gga ttt gga tat gaa ttc gag aga ttt cgt gta gat     1104
Ala Phe Thr Thr Gly Phe Gly Tyr Glu Phe Glu Arg Phe Arg Val Asp
        355                 360                 365

gcc atg gcg gaa tat tta cta gga aac gaa aga agc ttc cac aat aca     1152
Ala Met Ala Glu Tyr Leu Leu Gly Asn Glu Arg Ser Phe His Asn Thr
    370                 375                 380

caa tat aac ttt ggg ggc gac atc aac act ggt ggc tat gtg ttt ggt     1200
Gln Tyr Asn Phe Gly Gly Asp Ile Asn Thr Gly Gly Tyr Val Phe Gly
385                 390                 395                 400

cta ggt tta tcg tat aga ctt gac aaa taa                            1230
Leu Gly Leu Ser Tyr Arg Leu Asp Lys
                405
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<210> 10
<211> 409
<212> PRT
<213> *Ornithobacterium rhinotracheale*

<400> 10

Met Ile Lys Lys Val Phe Leu Ser Phe Val Leu Met Ala Ser Thr Gly
1 5 10 15

Ile Leu Trp Ala Gly Gly Tyr Arg Val Ser Leu Gln Gly Val Arg Gln
20 25 30

Ala Ala Met Gly Ala Gln Gly Val Ala Leu Ser His Asp Ala Ser Val
35 40 45

Ala Phe Phe Asn Pro Ala Ala Leu Ala Phe Val Asp Asp Lys Leu Ser
50 55 60

Ile Ala Val Gly Gly Phe Gly Ile Gly Ile Thr Ala Lys Tyr Gln Asn
65 70 75 80

Arg Glu Thr Leu Tyr Lys Ala Glu Thr Asp Asn Pro Leu Gly Thr Pro
85 90 95

Leu Tyr Leu Ala Thr Ser Tyr Lys Pro Thr Glu Lys Leu Ala Leu Gly
100 105 110

Val Ser Val Thr Thr Pro Phe Gly Ser Thr Val Asp Trp Gly Asp Lys
115 120 125

Trp Ala Gly Arg Tyr Ile Ile Asp Arg Ile Ala Leu Lys Ser Phe Phe
130 135 140

Ile Gln Pro Thr Ala Ala Tyr Lys Val Thr Asp Trp Leu Ser Val Gly
145 150 155 160

Ala Gly Ala Ile Ile Ala Arg Gly Asn Val Asn Ile Lys Arg Ala Ile
165 170 175

Ser Leu Gly Asn Gln Asp Ala Gly Leu Glu Ile Asp Lys Lys Gly Ala
180 185 190

His Gly Thr Gly Phe Asn Val Gly Val Tyr Ala Lys Pro Asn Asp Lys
195 200 205

Leu Asn Ile Gly Ile Ala Tyr Arg Ser Glu Val Lys Met Lys Ala Asp
210 215 220

Lys Gly Asp Ala Val Phe Lys Asn Leu Pro Ser Ile Val Lys Gly Lys
225 230 235 240

Met Pro Phe Ser Ala Lys Tyr Phe Asp Ala Gln Leu Pro Leu Pro Ala
245 250 255

Glu Leu Leu Ile Gly Ala Asn Tyr Lys Val Thr Pro Lys Leu Leu Val
260 265 270

Gly Ala Glu Ile Gly Ala Val Lys Trp Asn Ala Tyr Glu Thr Leu Asn
275 280 285

Ile Lys Leu Tyr Asn Asn Glu Glu Glu Tyr Asn Asn Thr Ser Asn Lys
290 295 300

Asn Tyr Lys Asn Thr Leu Asn Tyr Ser Ile Gly Ala Glu Tyr Leu Ile
305 310 315 320

Asn Pro Lys Ala Ala Leu Arg Leu Gly Tyr Lys Phe Asp Lys Ser Pro
325 330 335

Ser Pro Ala Asp Ser Phe Asn Pro Glu Thr Pro Thr Ile Asn Tyr His
340 345 350

Ala Phe Thr Thr Gly Phe Gly Tyr Glu Phe Glu Arg Phe Arg Val Asp
355 360 365

Ala Met Ala Glu Tyr Leu Leu Gly Asn Glu Arg Ser Phe His Asn Thr
370 375 380

Gln Tyr Asn Phe Gly Gly Asp Ile Asn Thr Gly Gly Tyr Val Phe Gly
385 390 395 400

Leu Gly Leu Ser Tyr Arg Leu Asp Lys
405

<210> 11
<211> 1140
<212> DNA
<213> *Ornithobacterium rhinotracheale*

<220>
<221> CDS
<222> (1)..(1140)

<400> 11
atg aag aaa ata ctt tta gca att agc ttt tcg tct ttt gtt tta agc 48
Met Lys Lys Ile Leu Leu Ala Ile Ser Phe Ser Ser Phe Val Leu Ser
1 5 10 15

tgt agc agt gat gat tac act cca gcc aca cct aaa gaa aca gaa aag 96
Cys Ser Ser Asp Asp Tyr Thr Pro Ala Thr Pro Lys Glu Thr Glu Lys
20 25 30

cct aag gaa gag gct gtg gtt cca aat aag cca gat gaa cca aag gct 144
Pro Lys Glu Glu Ala Val Val Pro Asn Lys Pro Asp Glu Pro Lys Ala
35 40 45

gat gat gga aac gaa aat cca gaa aac act gga gat gaa gag aat gga 192

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Asp Asp Gly Asn Glu Asn Pro Glu Asn Thr Gly Asp Glu Glu Asn Gly
    50                  55                  60

gat aat aca aac tcc gtt gtc ggg aag cct gat gat ttc cac atg ggg      240
Asp Asn Thr Asn Ser Val Val Gly Lys Pro Asp Asp Phe His Met Gly
65                  70                  75                  80

aat cgc tct tat gct agc tgg aaa gaa gat gtg gat tat atc gga ggt      288
Asn Arg Ser Tyr Ala Ser Trp Lys Glu Asp Val Asp Tyr Ile Gly Gly
                85                  90                  95

ttt gat att gaa act ctt tta agt ggg gct gat aat caa aaa tat gat      336
Phe Asp Ile Glu Thr Leu Leu Ser Gly Ala Asp Asn Gln Lys Tyr Asp
            100                 105                 110

gcg gct tat ttt agc caa ttt atc aag ata ttc tca tct agt cca aac      384
Ala Ala Tyr Phe Ser Gln Phe Ile Lys Ile Phe Ser Ser Ser Pro Asn
        115                 120                 125

gga aac aat ttc tac act ttt cag gca gaa gac ttt aaa gat gtc gag      432
Gly Asn Asn Phe Tyr Thr Phe Gln Ala Glu Asp Phe Lys Asp Val Glu
    130                 135                 140

att aaa gac tta aag ttt gat att ggt aga aat gta att aca ttt aaa      480
Ile Lys Asp Leu Lys Phe Asp Ile Gly Arg Asn Val Ile Thr Phe Lys
145                 150                 155                 160

act agc tac aaa ggc gta aaa agt gaa att aca tct tct tta aaa ttt      528
Thr Ser Tyr Lys Gly Val Lys Ser Glu Ile Thr Ser Ser Leu Lys Phe
                165                 170                 175

gat ttg gct aat ttt tat gat cga aaa atc aaa ata aac gaa gat ttc      576
Asp Leu Ala Asn Phe Tyr Asp Arg Lys Ile Lys Ile Asn Glu Asp Phe
            180                 185                 190

gtt gca tct cac tac atg aga ggg att tat gag gag ctt gga ggt ttt      624
Val Ala Ser His Tyr Met Arg Gly Ile Tyr Glu Glu Leu Gly Gly Phe
        195                 200                 205

atc ggg aat tta tta aac tac gac gat gag aaa tac aat cta gag tta      672
Ile Gly Asn Leu Leu Asn Tyr Asp Asp Glu Lys Tyr Asn Leu Glu Leu
    210                 215                 220

gcg ggg tca aaa aac aaa gat gaa tcc aat aac tct tta ggt ttt agc      720
Ala Gly Ser Lys Asn Lys Asp Glu Ser Asn Asn Ser Leu Gly Phe Ser
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225 230 235 240

att cgc gta aca gat aaa aaa gat aag tat ata aca acg gtt tat aaa 768
Ile Arg Val Thr Asp Lys Lys Asp Lys Tyr Ile Thr Thr Val Tyr Lys
245 250 255

aac atc tca gga ttt agg cct ctt tct agt ctg cag gag gag ctt tcc 816
Asn Ile Ser Gly Phe Arg Pro Leu Ser Ser Leu Gln Glu Glu Leu Ser
260 265 270

att gct cct act tac gaa ttg cga gag aaa atc aag gag aaa ata gat 864
Ile Ala Pro Thr Tyr Glu Leu Arg Glu Lys Ile Lys Glu Lys Ile Asp
275 280 285

aga aat aaa aga aac att agc cta ttg gag cta tta aaa cca tcg gta 912
Arg Asn Lys Arg Asn Ile Ser Leu Leu Glu Leu Leu Lys Pro Ser Val
290 295 300

aac gaa tgg atg aag tct gcc gat ttc tac ttt aat aac act gat ttg 960
Asn Glu Trp Met Lys Ser Ala Asp Phe Tyr Phe Asn Asn Thr Asp Leu
305 310 315 320

gaa tgg aga gga gat cat tat tca gct aga ggg ttt tta gat ttg tat 1008
Glu Trp Arg Gly Asp His Tyr Ser Ala Arg Gly Phe Leu Asp Leu Tyr
325 330 335

ata ggt tcg cct aga ttt gag ctg att tta gca aca aaa gaa gac aat 1056
Ile Gly Ser Pro Arg Phe Glu Leu Ile Leu Ala Thr Lys Glu Asp Asn
340 345 350

tgg ttg att ttg aaa gtg aaa gtg gtt cag ata aat gaa gtg cct acc 1104
Trp Leu Ile Leu Lys Val Lys Val Val Gln Ile Asn Glu Val Pro Thr
355 360 365

gat ttg gtg tat agc tta aga gtt tca att aac taa 1140
Asp Leu Val Tyr Ser Leu Arg Val Ser Ile Asn
370 375

<210> 12
<211> 379
<212> PRT
<213> *Ornithobacterium rhinotracheale*

<400> 12

Met Lys Lys Ile Leu Leu Ala Ile Ser Phe Ser Ser Phe Val Leu Ser
1 5 10 15

Cys Ser Ser Asp Asp Tyr Thr Pro Ala Thr Pro Lys Glu Thr Glu Lys
20 25 30

Pro Lys Glu Glu Ala Val Val Pro Asn Lys Pro Asp Glu Pro Lys Ala
35 40 45

Asp Asp Gly Asn Glu Asn Pro Glu Asn Thr Gly Asp Glu Glu Asn Gly
50 55 60

Asp Asn Thr Asn Ser Val Val Gly Lys Pro Asp Asp Phe His Met Gly
65 70 75 80

Asn Arg Ser Tyr Ala Ser Trp Lys Glu Asp Val Asp Tyr Ile Gly Gly
85 90 95

Phe Asp Ile Glu Thr Leu Leu Ser Gly Ala Asp Asn Gln Lys Tyr Asp
100 105 110

Ala Ala Tyr Phe Ser Gln Phe Ile Lys Ile Phe Ser Ser Ser Pro Asn
115 120 125

Gly Asn Asn Phe Tyr Thr Phe Gln Ala Glu Asp Phe Lys Asp Val Glu
130 135 140

Ile Lys Asp Leu Lys Phe Asp Ile Gly Arg Asn Val Ile Thr Phe Lys
145 150 155 160

Thr Ser Tyr Lys Gly Val Lys Ser Glu Ile Thr Ser Ser Leu Lys Phe
165 170 175

Asp Leu Ala Asn Phe Tyr Asp Arg Lys Ile Lys Ile Asn Glu Asp Phe
180 185 190

Val Ala Ser His Tyr Met Arg Gly Ile Tyr Glu Glu Leu Gly Gly Phe
195 200 205

Ile Gly Asn Leu Leu Asn Tyr Asp Asp Glu Lys Tyr Asn Leu Glu Leu
210 215 220

Ala Gly Ser Lys Asn Lys Asp Glu Ser Asn Asn Ser Leu Gly Phe Ser
225 230 235 240

Ile Arg Val Thr Asp Lys Lys Asp Lys Tyr Ile Thr Thr Val Tyr Lys
245 250 255

Asn Ile Ser Gly Phe Arg Pro Leu Ser Ser Leu Gln Glu Glu Leu Ser
260 265 270

Ile Ala Pro Thr Tyr Glu Leu Arg Glu Lys Ile Lys Glu Lys Ile Asp
275 280 285

Arg Asn Lys Arg Asn Ile Ser Leu Leu Glu Leu Leu Lys Pro Ser Val
290 295 300

Asn Glu Trp Met Lys Ser Ala Asp Phe Tyr Phe Asn Asn Thr Asp Leu
305 310 315 320

Glu Trp Arg Gly Asp His Tyr Ser Ala Arg Gly Phe Leu Asp Leu Tyr
325 330 335

Ile Gly Ser Pro Arg Phe Glu Leu Ile Leu Ala Thr Lys Glu Asp Asn
340 345 350

Trp Leu Ile Leu Lys Val Lys Val Val Gln Ile Asn Glu Val Pro Thr

355 360 365

Asp Leu Val Tyr Ser Leu Arg Val Ser Ile Asn
370 375

<210> 13
<211> 918
<212> DNA
<213> *Ornithobacterium rhinotracheale*

<220>
<221> CDS
<222> (1)..(918)

<400> 13

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atg att gta aaa gac ttt tca gac tat aca ttc cga tgt tct caa tta      48
Met Ile Val Lys Asp Phe Ser Asp Tyr Thr Phe Arg Cys Ser Gln Leu
1               5                  10                  15

ggt aag tta atg gtt ggt gtc aag cca cca tta acc cct aat caa gag      96
Gly Lys Leu Met Val Gly Val Lys Pro Pro Leu Thr Pro Asn Gln Glu
            20                  25                  30

aag ttg ctc aca gac tta gag ggc aaa atg gaa gct ggg acc att acc     144
Lys Leu Leu Thr Asp Leu Glu Gly Lys Met Glu Ala Gly Thr Ile Thr
        35                  40                  45

aaa aag caa atc atc act tat ggt gaa ttg ctt tcc aag aaa aac caa     192
Lys Lys Gln Ile Ile Thr Tyr Gly Glu Leu Leu Ser Lys Lys Asn Gln
    50                  55                  60

aag ctt gaa tta tct gca agt gta aag tct tac tta gcc gac att cat     240
Lys Leu Glu Leu Ser Ala Ser Val Lys Ser Tyr Leu Ala Asp Ile His
65                  70                  75                  80

aaa gaa gtc ttt ttt ggt cgt gat aag gaa ttg acc aat aaa tat cta     288
Lys Glu Val Phe Phe Gly Arg Asp Lys Glu Leu Thr Asn Lys Tyr Leu
                85                  90                  95

tca aaa ggc att caa gta gaa gaa aag agc ata acg ctc tat tcc gat     336
Ser Lys Gly Ile Gln Val Glu Glu Lys Ser Ile Thr Leu Tyr Ser Asp
            100                 105                 110
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gtc tgt aac aag tta ttc cta aag aat aaa aag ttt tac aaa aac gat 384
Val Cys Asn Lys Leu Phe Leu Lys Asn Lys Lys Phe Tyr Lys Asn Asp
115 120 125

ttt att caa ggt acg cca gat aac acg caa gac aaa atc aga gat atc 432
Phe Ile Gln Gly Thr Pro Asp Asn Thr Gln Asp Lys Ile Arg Asp Ile
130 135 140

aaa agt agt tgg gac ttc tca acc ttt cct cta cac gcc gat gaa acg 480
Lys Ser Ser Trp Asp Phe Ser Thr Phe Pro Leu His Ala Asp Glu Thr
145 150 155 160

cca acc aaa gac tat gaa tgg cag ttg caa ggt tat atg gaa tta aca 528
Pro Thr Lys Asp Tyr Glu Trp Gln Leu Gln Gly Tyr Met Glu Leu Thr
165 170 175

ggc tta aaa gaa gct gag ttg att tat tgc ttg gtt gat acg cct cat 576
Gly Leu Lys Glu Ala Glu Leu Ile Tyr Cys Leu Val Asp Thr Pro His
180 185 190

aaa att gta gaa gat gaa atc cga aga atg gac tgg aag cat aat tta 624
Lys Ile Val Glu Asp Glu Ile Arg Arg Met Asp Trp Lys His Asn Leu
195 200 205

ctt gac att aac ggc gaa gtg aga gcc gag aca aga gat tta gta gtt 672
Leu Asp Ile Asn Gly Glu Val Arg Ala Glu Thr Arg Asp Leu Val Val
210 215 220

gag att gtg tct aac tta att tat acc aag caa ggc ttg gaa gac ttt 720
Glu Ile Val Ser Asn Leu Ile Tyr Thr Lys Gln Gly Leu Glu Asp Phe
225 230 235 240

tgt cag cag tcc gca gtc ata aac aaa gat tgg ttc acg gac ttt gag 768
Cys Gln Gln Ser Ala Val Ile Asn Lys Asp Trp Phe Thr Asp Phe Glu
245 250 255

gaa ata cca caa gaa ttg aga att aaa gtt ttt cac ttt gag cat caa 816
Glu Ile Pro Gln Glu Leu Arg Ile Lys Val Phe His Phe Glu His Gln
260 265 270

aaa gag atg att agc gca ctc tac gag caa ata gga aga tgt aga gcg 864
Lys Glu Met Ile Ser Ala Leu Tyr Glu Gln Ile Gly Arg Cys Arg Ala
275 280 285

cat tta aac gac ttg acc atg aaa atg gca aca cga tta gaa tta ata 912
His Leu Asn Asp Leu Thr Met Lys Met Ala Thr Arg Leu Glu Leu Ile
290 295 300

gca taa 918
Ala
305

<210> 14
<211> 305
<212> PRT
<213> *Ornithobacterium rhinotracheale*

<400> 14

Met Ile Val Lys Asp Phe Ser Asp Tyr Thr Phe Arg Cys Ser Gln Leu
1 5 10 15

Gly Lys Leu Met Val Gly Val Lys Pro Pro Leu Thr Pro Asn Gln Glu
20 25 30

Lys Leu Leu Thr Asp Leu Glu Gly Lys Met Glu Ala Gly Thr Ile Thr
35 40 45

Lys Lys Gln Ile Ile Thr Tyr Gly Glu Leu Leu Ser Lys Lys Asn Gln
50 55 60

Lys Leu Glu Leu Ser Ala Ser Val Lys Ser Tyr Leu Ala Asp Ile His
65 70 75 80

Lys Glu Val Phe Phe Gly Arg Asp Lys Glu Leu Thr Asn Lys Tyr Leu
85 90 95

Ser Lys Gly Ile Gln Val Glu Glu Lys Ser Ile Thr Leu Tyr Ser Asp
100 105 110

Val Cys Asn Lys Leu Phe Leu Lys Asn Lys Lys Phe Tyr Lys Asn Asp

115 120 125

Phe Ile Gln Gly Thr Pro Asp Asn Thr Gln Asp Lys Ile Arg Asp Ile
130 135 140

Lys Ser Ser Trp Asp Phe Ser Thr Phe Pro Leu His Ala Asp Glu Thr
145 150 155 160

Pro Thr Lys Asp Tyr Glu Trp Gln Leu Gln Gly Tyr Met Glu Leu Thr
165 170 175

Gly Leu Lys Glu Ala Glu Leu Ile Tyr Cys Leu Val Asp Thr Pro His
180 185 190

Lys Ile Val Glu Asp Glu Ile Arg Arg Met Asp Trp Lys His Asn Leu
195 200 205

Leu Asp Ile Asn Gly Glu Val Arg Ala Glu Thr Arg Asp Leu Val Val
210 215 220

Glu Ile Val Ser Asn Leu Ile Tyr Thr Lys Gln Gly Leu Glu Asp Phe
225 230 235 240

Cys Gln Gln Ser Ala Val Ile Asn Lys Asp Trp Phe Thr Asp Phe Glu
245 250 255

Glu Ile Pro Gln Glu Leu Arg Ile Lys Val Phe His Phe Glu His Gln
260 265 270

Lys Glu Met Ile Ser Ala Leu Tyr Glu Gln Ile Gly Arg Cys Arg Ala
275 280 285

His Leu Asn Asp Leu Thr Met Lys Met Ala Thr Arg Leu Glu Leu Ile
290 295 300

Ala
305

<210> 15
<211> 888
<212> DNA
<213> *Ornithobacterium rhinotracheale*

<220>
<221> CDS
<222> (1)..(888)

<400> 15

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atg aac gaa tta gcg aaa aac gac atc aag tca ttg tta aaa agt gcc      48
Met Asn Glu Leu Ala Lys Asn Asp Ile Lys Ser Leu Leu Lys Ser Ala
1               5                   10                  15

gac atc aac aaa aga ttt gag caa ttg ctc ggc aaa aaa gca caa ggc      96
Asp Ile Asn Lys Arg Phe Glu Gln Leu Leu Gly Lys Lys Ala Gln Gly
            20                  25                  30

ttt atc tca tca gtc ttg cag acg gca caa aat aac aga ttg tta gcg     144
Phe Ile Ser Ser Val Leu Gln Thr Ala Gln Asn Asn Arg Leu Leu Ala
        35                  40                  45

aca gcc gac cca aag acc att cta aac gct gca gta aca gcc gcg act     192
Thr Ala Asp Pro Lys Thr Ile Leu Asn Ala Ala Val Thr Ala Ala Thr
    50                  55                  60

tta gat ttg cca att aat cag aat tta ggt tac gcc tac atc gtg cct     240
Leu Asp Leu Pro Ile Asn Gln Asn Leu Gly Tyr Ala Tyr Ile Val Pro
65                  70                  75                  80

tac aaa ggg cag gcg caa ttc caa tta ggc tgg aag ggc ttt gta gca     288
Tyr Lys Gly Gln Ala Gln Phe Gln Leu Gly Trp Lys Gly Phe Val Ala
                85                  90                  95

tta gct aaa aga agt ggc gca tat ttg aaa atg aat gta gta act gtc     336
Leu Ala Lys Arg Ser Gly Ala Tyr Leu Lys Met Asn Val Val Thr Val
            100                 105                 110
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tat caa aat caa ttc aaa tcc tac aat cgc tta aca gaa gaa tta gat 384
Tyr Gln Asn Gln Phe Lys Ser Tyr Asn Arg Leu Thr Glu Glu Leu Asp
115 120 125

gcc gat ttc aca atc gaa ggc aat ggt gaa gta gtt ggt tat gca gcc 432
Ala Asp Phe Thr Ile Glu Gly Asn Gly Glu Val Val Gly Tyr Ala Ala
130 135 140

tat ttc aaa gaa atc aat ggt ttt gaa aag ctt tcg ttt tgg tca att 480
Tyr Phe Lys Glu Ile Asn Gly Phe Glu Lys Leu Ser Phe Trp Ser Ile
145 150 155 160

gag caa gta aaa aaa cac gcc acc aaa tac tct caa act tat ggt aaa 528
Glu Gln Val Lys Lys His Ala Thr Lys Tyr Ser Gln Thr Tyr Gly Lys
165 170 175

aaa tca cgc tcg ggg gca tta atg ttt tcg cct tgg aat gat gaa gac 576
Lys Ser Arg Ser Gly Ala Leu Met Phe Ser Pro Trp Asn Asp Glu Asp
180 185 190

cag ttt gac gca atg gct atg aag act gtc tta aaa aac acg ctc tca 624
Gln Phe Asp Ala Met Ala Met Lys Thr Val Leu Lys Asn Thr Leu Ser
195 200 205

aag ttt ggg aca ctc tca att gaa atg caa atg gcg caa atg gca gac 672
Lys Phe Gly Thr Leu Ser Ile Glu Met Gln Met Ala Gln Met Ala Asp
210 215 220

caa gca gtc atc aag aac gag ggg gag tac gag tat ata gac aat acc 720
Gln Ala Val Ile Lys Asn Glu Gly Glu Tyr Glu Tyr Ile Asp Asn Thr
225 230 235 240

ata gac att gaa gct gaa agt gcc gaa gaa gaa gcc aat cgt att atg 768
Ile Asp Ile Glu Ala Glu Ser Ala Glu Glu Glu Ala Asn Arg Ile Met
245 250 255

aaa ttt att gat aaa gcc gaa agc att gaa gcc tta gag gaa tta aaa 816
Lys Phe Ile Asp Lys Ala Glu Ser Ile Glu Ala Leu Glu Glu Leu Lys
260 265 270

tca tca gtt gat gag aat ggc gat tta gag tta tta gcc tat tac gac 864
Ser Ser Val Asp Glu Asn Gly Asp Leu Glu Leu Leu Ala Tyr Tyr Asp
275 280 285

aac aga aaa aat gaa tta aaa tga 888

Asn Arg Lys Asn Glu Leu Lys
290 295

<210> 16
<211> 295
<212> PRT
<213> *Ornithobacterium rhinotracheale*

<400> 16

Met Asn Glu Leu Ala Lys Asn Asp Ile Lys Ser Leu Leu Lys Ser Ala
1 5 10 15

Asp Ile Asn Lys Arg Phe Glu Gln Leu Leu Gly Lys Lys Ala Gln Gly
20 25 30

Phe Ile Ser Ser Val Leu Gln Thr Ala Gln Asn Asn Arg Leu Leu Ala
35 40 45

Thr Ala Asp Pro Lys Thr Ile Leu Asn Ala Ala Val Thr Ala Ala Thr
50 55 60

Leu Asp Leu Pro Ile Asn Gln Asn Leu Gly Tyr Ala Tyr Ile Val Pro
65 70 75 80

Tyr Lys Gly Gln Ala Gln Phe Gln Leu Gly Trp Lys Gly Phe Val Ala
85 90 95

Leu Ala Lys Arg Ser Gly Ala Tyr Leu Lys Met Asn Val Val Thr Val
100 105 110

Tyr Gln Asn Gln Phe Lys Ser Tyr Asn Arg Leu Thr Glu Glu Leu Asp
115 120 125

Ala Asp Phe Thr Ile Glu Gly Asn Gly Glu Val Val Gly Tyr Ala Ala
130 135 140

Tyr Phe Lys Glu Ile Asn Gly Phe Glu Lys Leu Ser Phe Trp Ser Ile
145 150 155 160

Glu Gln Val Lys Lys His Ala Thr Lys Tyr Ser Gln Thr Tyr Gly Lys
165 170 175

Lys Ser Arg Ser Gly Ala Leu Met Phe Ser Pro Trp Asn Asp Glu Asp
180 185 190

Gln Phe Asp Ala Met Ala Met Lys Thr Val Leu Lys Asn Thr Leu Ser
195 200 205

Lys Phe Gly Thr Leu Ser Ile Glu Met Gln Met Ala Gln Met Ala Asp
210 215 220

Gln Ala Val Ile Lys Asn Glu Gly Glu Tyr Glu Tyr Ile Asp Asn Thr
225 230 235 240

Ile Asp Ile Glu Ala Glu Ser Ala Glu Glu Glu Ala Asn Arg Ile Met
245 250 255

Lys Phe Ile Asp Lys Ala Glu Ser Ile Glu Ala Leu Glu Glu Leu Lys
260 265 270

Ser Ser Val Asp Glu Asn Gly Asp Leu Glu Leu Leu Ala Tyr Tyr Asp
275 280 285

Asn Arg Lys Asn Glu Leu Lys
290 295